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Method for the preparation of intermediates for carboxy-fluoresceins and novel carboxy-fluorescein.

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(54) **Title**: METHOD FOR THE PREPARATION OF INTERMEDIATES FOR CARBOXY-FLUORESCEINS AND NOVEL CARBOXY-FLUORESCEIN

(57) **Abstract**: The invention provides a method for the preparation of regioisomerically pure intermediates which are useful for the preparation of carboxy-fluorescein-type compounds. Such compounds have broad applications within bio-conjugation and/or fluorescent imaging.

METHOD FOR THE PREPARATION OF INTERMEDIATES FOR CARBOXY-FLUORESCEINS AND NOVEL CARBOXY-FLUORESCEIN

FIELD OF THE INVENTION

The present invention relates to a novel method for the preparation of regioisomerically pure intermediates which are useful for the preparation of carboxy-fluorescein-type compounds. Such compounds have broad applications within bio-conjugation and/or fluorescent imaging.

BACKGROUND OF THE INVENTION

5(6)-Carboxy-fluorescein is a well-known chromophore and mixtures of the two regioisomers can with great effort be separated into the pure regioisomers 5- and 6-carboxyfluorescein by HPLC. Burgess and co-workers [Y. Ueno, G.-S. Jiao, K. Burgess, *Synthesis (Stuttg).* **2004**, 2591–2593] have reported a procedure using fractional crystallization in multi-gram amounts with 98 % regioisomeric purity of both isomers. Separation of other regioisomeric derivatives of fluorescein has also been achieved [F. M. Rossi, J. P. Kao, *Bioconjugate Chem.* **1997**, *8*, 495–497; G.-S. Jiao, J. W. Han, K. Burgess, *J. Org. Chem.* **2003**, *68*, 8264–8267; M. Adamczyk, C. M. Chan, J. R. Fino, P. G. Mattingly, *J. Org. Chem.* **2000**, *68*, 596–601; C. C. Woodroofe, M. H. Lim, W. Bu, S. J. Lippard, *Tetrahedron* **2005**, *61*, 3097–3105.]. However, to our knowledge, a large scale synthesis without chromatographic purification to produce 100 % regioisomerically pure carboxyfluoresceins has never been disclosed.

US 2002/146726 A1 discloses electrophoretic tag reagents comprising fluorescent compounds.

CN 103 012 354 A seems to disclose a method for the preparation og 5- and 6-carboxyfluorescein.

US 4 945 171 A discloses xanthene dyes having a fused (c) benzo ring.

Sikhibhushan Dutt: CL. – A theory of Colour on the Basis of Molecular Strain. The Effect of Chromophoric Superposition, J. Chem. Soc. Vol 129, Jan 1926 (1926-01), p. 1171-1184, XP55136205, ISSN: 0368-1769, DOI: 10.1039/jr9262901171 discloses a number of chromophores.

US 6 229 024 B1 discloses a method for detecting neuronal degeneration and anionic fluorescein homologue stains therefor.

US 8 029 765 B2 discloses SMMR (Small Molecule Metabolite Reporters) for use as in vivo glucose biosensors.

US 5 800 996 A discloses energy transfer dyes with enhanced fluorescence.

It is an object of embodiments of the invention to provide a method for the easy and cost efficient synthesis of regioisomerically pure key intermediates which are useful for the preparation of a variety of carboxy-fluoresceins including carboxy-SNAFL derivatives. By providing a method for the preparation of key intermediates which are regioisomerically pure a simple and efficient production suitable for large scale synthesis of a variety of carboxy-fluoresceins have become possible.

SUMMARY OF THE INVENTION

It has been found by the present inventor(s) that the benzophenones 4-(2,4-dihydroxybenzoyl)isophthalic acid (**6**) and 2-(2,4-dihydroxybenzoyl)terephthalic acid (**5**) can be prepared in high regioisomerical purity by condensation of trimellitic anhydride with resorcinol with subsequent partial reversal of the condensation by hydrolysis under basic conditions, followed by acidification, isolation and fractional crystallisation of each of the target compounds.

So, in a first aspect the present invention relates to the methods defined in claim 1 and in claim 2.

In a second aspect, the invention relates to the novel carboxy-fluorescein derivatives defined in claims 12-15.

In a third aspect, the invention relates to the novel intermediates **5** and **6** defined in claim 16.

BRIEF DESCRIPTION OF THE SCHEMES

Scheme 1. Synthetic route to regioisomerically pure 5- and 6-carboxyfluorescein (**7** and **8**) and mixed fluorescein derivatives **9**-**11**.

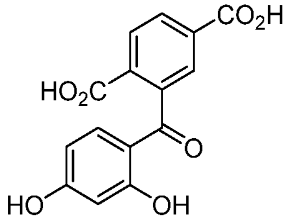
Scheme 2. Synthetic route to mixed difluorescein derivatives **14**-**18**.

Scheme 3. Synthesis of type [a], [b] and [c]benzoxanthenes.

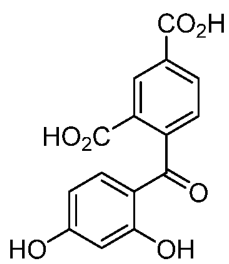
DETAILED DISCLOSURE OF THE INVENTION

*Method for the preparation and isolation of compound **5** and compound **6***

One aspect of the invention relates to a method for the preparation and isolating of compound **6** and, optionally, of compound **5**. The method is illustrated generally in Scheme 1.



5



6.

It should be understood that the method is useful for the preparation and isolation of both compounds, but insofar that only compound **6** is of interest, compound **5** need not be isolated.

Step (i)

In the first step of the method, a condensation product mixture is provided, being the result of a condensation reaction between trimellitic anhydride and resorcinol mediated by acid. The condensation product mixture comprises a mixture of crude 5- and 6-carboxy-fluorescein.

The method can begin from the condensation product mixture itself, or include a pre-step, in which trimellitic anhydride is reacted with resorcinol in a strong acid so as to obtain the condensation product mixture. Although not strictly necessary, the condensation product mixture is typically worked up by pouring the reaction mixture into cold water (e.g. ice water), isolation of the solid mater by filtration, refluxing in EtOH, and re-precipitation by addition of water, whereby a mixture of crude 5- and 6-carboxy-fluorescein is obtained.

Examples of acids suitable for the acid-mediated condensation reaction are methanesulfonic acid (MSA), mixtures of methanesulfonic acid and trifluoroacetic acid (TFA), e.g. an approx. 1:1 mixture of MSA and TFA, and $ZnCl_2$. Methanesulfonic acid is a currently preferred choice. Alternative strong acids include H_2SO_4, $SnCl_4$, acetic acid, H_3PO_4, HF, BF_3 and BBr_3.

The condensation reaction is conducted as previously described in the literature. Hence, typical conditions are reaction for 10-40 hours at 50-100°C, either with or without an inert atmosphere.

Step (ii)

Subsequent to the condensation, the condensation product mixture (i.e. the crude 5- and 6-carboxy-fluorescein) is hydrolysed with a strong aqueous base at pH at least 11, typically at pH 12-14, so as to partly reverse the condensation reaction.

Examples of strong aqueous bases are 5:1/1:5 weight ratio of NaOH, KOH, LiOH, CsOH, $Ca(OH)_2$, $Ba(OH)_2$, $Sr(OH)_2$, NH_3 and H_2O of which 1:1 weight ratio of NaOH and H_2O is currently preferred. The skilled person will be able to select other strong aqueous bases which will achieve the desired result.

The hydrolysis is typically carried out from 1-200 hours, preferably 5-100hours, more preferably 12-48 hours. Typical temperatures for the hydrolysis are 0-150°C, preferably 40-100ºC.

In a most preferred combination of embodiments, hydrolysis is carried out using a 1:1 mixture of $NaOH/H_2O$ at 80 °C overnight.

Step (iii)

In a subsequent step, the reaction mixture of step (ii) is acidified so as to isolate a mixture of compound **5** and compound **6**.

Acidification is typically conducted by first pouring the hydrolysis reaction mixture into ice or cold water (ice water) after which a strong acid is slowly added until pH < 7. Examples of strong acids are HCl, H_3PO_4, H_2CO_3, H_2SO_4, acetic acid and HNO_3, of which 12 M HCl is currently preferred. The acidification is typically conducted at 0–10 °C. Acidification is usually carried out over a period of 1-4 hours.

Step (iv)

Subsequent to the acidification, the mixture of compound **5** and compound **6** is dissolved in methanol and water is then added so as to selectively precipitate compound **6**.

Crystallization is typically conducted at 0-30 °C, preferably 20 °C. Typical crystallisation times are 1-200 hours, preferably 24 hours. The solvent for recrystallization is typically 1-10 % v/v MeOH in H2O, preferably 5 % v/v.

Step (v)

In order to isolate compound **5** and any remaining compound **6**, the mother liquor from the crystallisation in step (iv) is extracted with an organic solvent, such as diethylether, ethyl acetate or dichloromethane. Of these, diethylether is preferred. The organic solvent is subsequently removed so as to obtain a dried extract. The extraction is conducted at room temperature, i.e. up to 25 °C.

Step (vi)

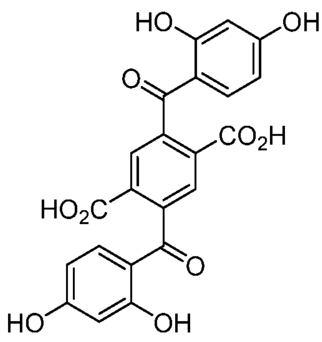
Steps (iv) and (v) may optionally be repeated in one or more additional cycles (e.g. 1-5 additional cycles) using the dried extract obtained in step (v) so as to crystallize out more of compound **6**. Typically, 2-3 additional cycles are preferred.

Step (vii)

Insofar as isolation of compound **5** is desirable, the dried extract obtained in step (v) is dissolved in refluxing H_2O and compound **5** is precipitated. Precipitation of compound **5** suitably takes place at 0-10 °C, in a time period of 1-200 hours, preferably 100 hours.

Method for the preparation and isolation of compound ***13***

Another aspect of the invention relates to a method for the preparation and isolation of compound **13**. The method is illustrated generally in Scheme 2.



13

Step (i)

In the first step of the method, a condensation product is provided, being the result of a condensation reaction between pyromellitic dianhydride and resorcinol in a strong acid.

The method begins with pyromellitic dianhydride that is reacted with resorcinol mediated by acid so as to obtain the condensation product. Although not strictly necessary, the condensation product is typically worked up by pouring the reaction mixture into cold water (e.g. ice water), isolation of the solid mater by filtration, refluxing in EtOH, and re-precipitation by addition of water, whereby the condensation product is obtained.

Examples of acids suitable for use in the condensation reaction are methanesulfonic acid (MSA), mixtures of methanesulfonic acid and trifluoroacetic acid (TFA), e.g. an approx. 1:1 mixture of MSA and TFA, and $ZnCl_2$. Methanesulfonic acid is a currently preferred choice. Alternative strong acids include H_2SO_4, $SnCl_4$, acetic acid,H_3PO_4, HF, BF_3 and BBr_3.

The condensation reaction is conducted as previously described in the literature. Hence, typical conditions are reaction for 10-40 hours at 50-100 °C, either with or without an inert atmosphere.

Step (ii)

Subsequent to the condensation, the condensation product is hydrolysed with a strong aqueous base at pH at least 11, typically at pH 12-14, so as to partly reverse the condensation reaction.

Examples of strong aqueous bases are 5:1/1:5 weight ratio of NaOH, KOH, LiOH, RbOH, $Ca(OH)_2$, $Ba(OH)_2$, $Sr(OH)_2$, NH_3 and H_2O of which 1:1 weight ratio of NaOH and H_2O is currently preferred. The skilled person will be able to select other strong aqueous bases which will achieve the desired result.

The hydrolysis is typically carried out from 1-200 hours, preferably 12-48 hours. Typical temperatures for the hydrolysis are 0-150 °C, preferably 40-100 ºC.

In a most preferred combination of embodiments, hydrolysis is carried out using a 1:1 (v/w) mixture of NaOH/H_2O at 80 °C overnight.

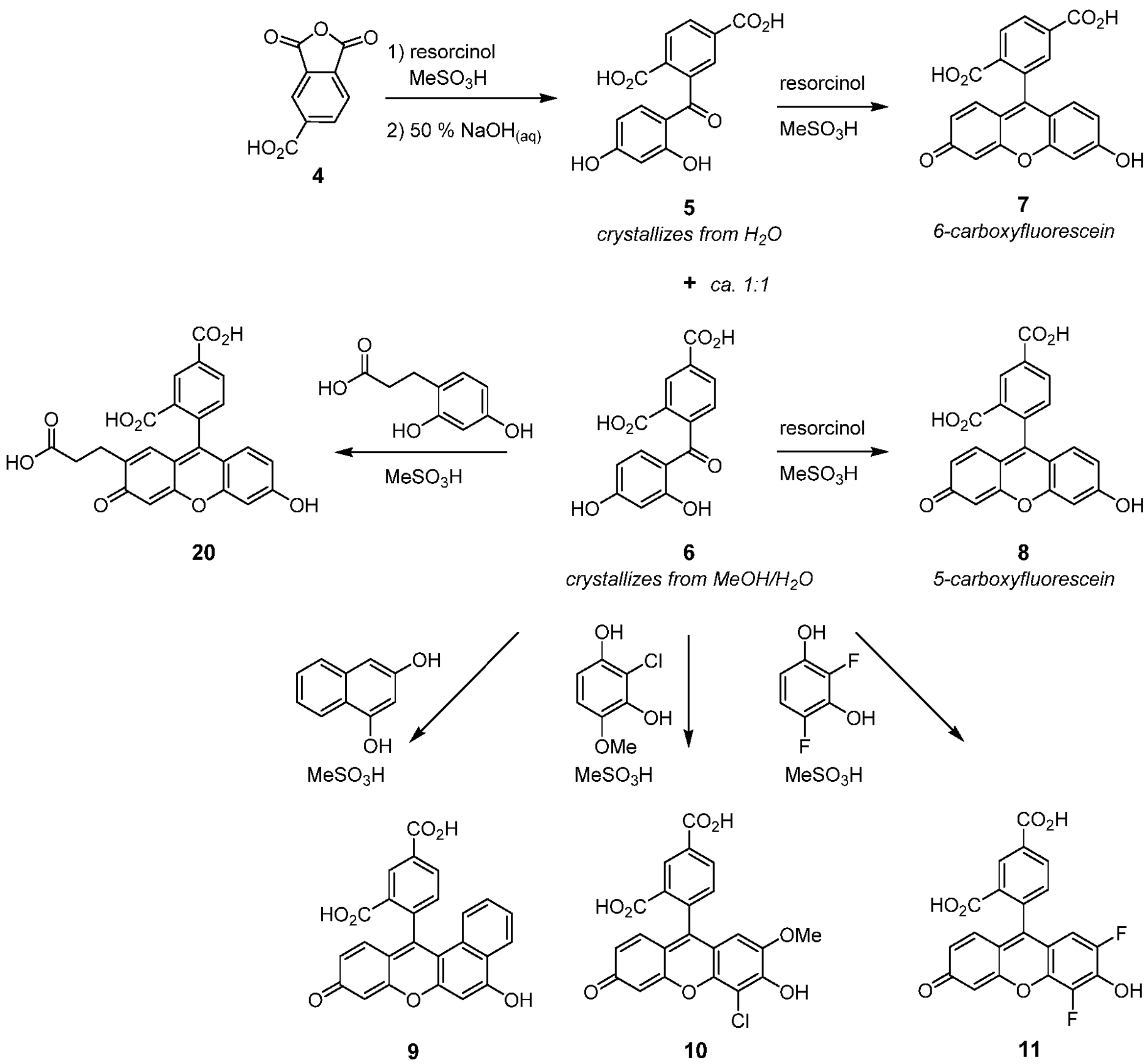
Step (iii)

In a subsequent step, the reaction mixture of step (ii) is acidified so as to isolate compound **13**.

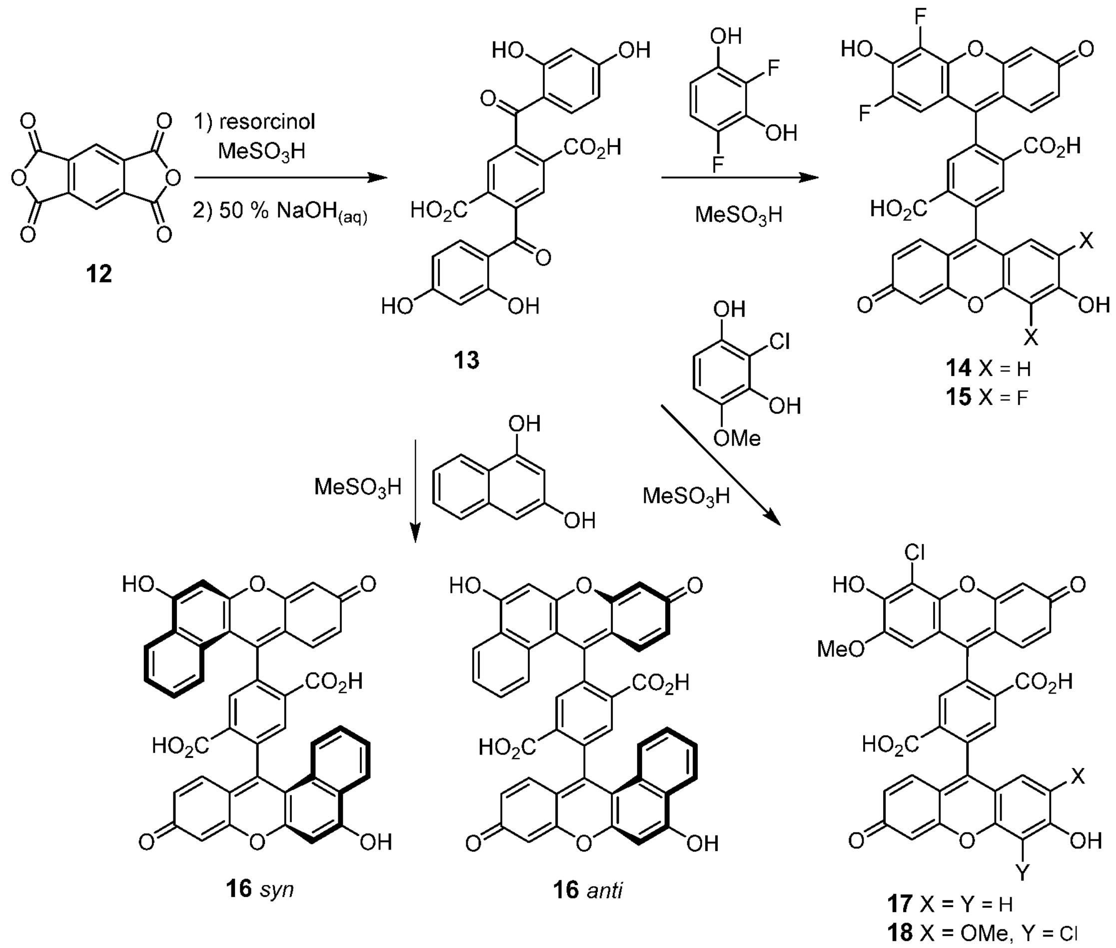
Acidification is typically conducted by first pouring the hydrolysis reaction mixture into ice or cold water (ice water) after which a strong acid is slowly added. Examples of strong acids are HCl, H_3PO_4, H_2CO_3, H_2SO_4, acetic acid and HNO_3, of which 12 M HCl is currently preferred. The acidification is typically conducted at 0–10 °C. Acidification is usually carried out over a period of 1-4 hours.

Method for the preparation of carboxy-fluoresceins

The compounds **5**, **6** and **13** prepared according to the methods described above are useful for the preparation of a broad range of carboxy-fluoresceins (see Schemes 1 and 2).

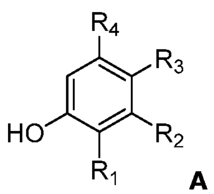

1) resorcinol
MeSO3H
2) 50 % NaOH(aq)
4
5
crystallizes from H2O
resorcinol
MeSO3H
7
6-carboxyfluorescein
+ ca. 1:1
MeSO3H
20
6
crystallizes from MeOH/H2O
resorcinol
MeSO3H
8
5-carboxyfluorescein
MeSO3H
MeSO3H
MeSO3H
9
10
11

Scheme 1

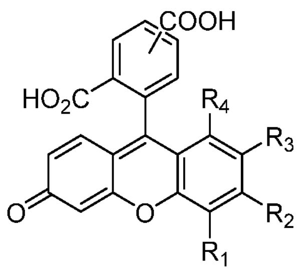


Scheme 2

Hence, the invention also provides a method wherein compound **5** or compound **6** (e.g. obtained as described further above) is subsequently reacted with a compound of formula **A**



in which R_1, R_2, R_3 and R_4 are independently selected from hydrogen; halogen; hydroxyl; nitro; cyano; mercapto; -O-C_{1-6}-alkyl; -S–C_{1-6}-alkyl; cyclopropyl; -C_{1-6}-alkyl; -C_{1-6}-alkyl-CONH-R_5, -C_{2-6}-alkenyl; or -C_{2-6}-alkynyl; which -O-C_{1-6}-alkyl, -S–C_{1-6}-alkyl, cyclopropyl, -C_{1-6}-alkyl, -C_{2-6}-alkenyl or -C_{2-6}-alkynyl is optionally substituted with at least one substituent selected from halogen, hydroxyl, -COOH, nitro, cyano and mercapto; wherein R_5 is selected from the group consisting of -C_{1-6}-alkyl and -$[CH_2CH_2O]_n$, wherein n=1-10,000, wherein said -C_{1-6}-alkyl and -$[CH_2CH_2O]_n$ are optionally substituted with a substituent selected from the group consisting of -NH-biotin, -C_{1-6}-alkyl-heterocycloalkyl, -DOTA, -NHCO-C_{1-6}-alkyl-heterocycloalkyl, -maleimide, -N_3, -C≡CH, -C-$_{1-6}$-alkyl-N_3, and -C-$_{1-6}$-alkyl-N(-C-$_{1-6}$-alkyl-heteroaryl)$_2$; with the additional option that any of the substituent pairs, R_1/R_2, R_2/R_3 and R_3/R_4 together with the intervening atoms may form an optionally substituted aromatic ring or ring system; in the presence of a strong acid (e.g. 99.5% pure methanesulfonic acid) so as to provide a compound of formula **B**

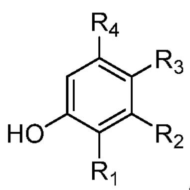


B

wherein R_1, R_2, R_3 and R_4 are as defined above.

Examples of strong acids suitable are methanesulfonic acid (MSA), mixtures of methanesulfonic acid and trifluoroacetic acid (TFA), e.g. an approx. 1:1 mixture of MSA and TFA, $ZnCl_2$. Methanesulfonic acid is a currently preferred choice. Alternative strong acids include H_2SO_4, $SnCl_4$, acetic acid,H_3PO_4, HF, BF_3 and BBr_3.

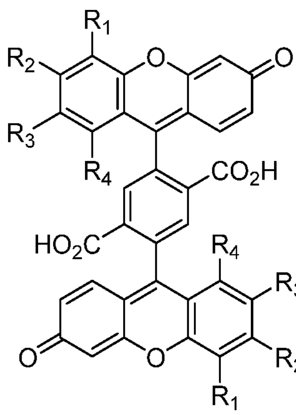
Also, the invention also provides a method wherein compound **13** is subsequently reacted with a compound of formula **A**



A

wherein R_1, R_2, R_3 and R_4 are independently selected from hydrogen; halogen; hydroxyl; nitro; cyano; mercapto; -O-C_{1-6}-alkyl; -S–C_{1-6}-alkyl; cyclopropyl; -C_{1-6}-alkyl; -C_{1-6}-alkyl-CONH-R_5, -C_{2-6}-alkenyl; or -C_{2-6}-alkynyl; which -O-C_{1-6}-alkyl, -S–C_{1-6}-alkyl, cyclopropyl, -C_{1-6}-alkyl, -C_{2-6}-alkenyl or -C_{2-6}-alkynyl is optionally substituted with at least one substituent selected from halogen, hydroxyl, -COOH, nitro, cyano and mercapto; wherein R_5 is selected from the group consisting of -C_{1-6}-alkyl and -$[CH_2CH_2O]_n$, wherein n=1-10,000, wherein said -C_{1-6}-alkyl and -$[CH_2CH_2O]_n$ are optionally substituted with a substituent selected from the group consisting of -NH-biotin, -C_{1-6}-alkyl-heterocycloalkyl, -DOTA, -NHCO-C_{1-6}-alkyl-heterocycloalkyl, -maleimide, -N_3, -C≡CH, -C-$_{1-6}$-alkyl-N_3, and -C-$_{1-6}$-alkyl-N(-C-$_{1-6}$-alkyl-heteroaryl)$_2$; with the additional option that any of the substituent pairs, R_1/R_2, R_2/R_3 and R_3/R_4 together with the intervening atoms may form an optionally substituted aromatic ring or ring system;

in the presence of a strong acid (e.g. 99.5% pure methanesulfonic acid) so as to provide a compound of formula **C**



C

wherein R_1, R_2, R_3 and R_4 are as defined above.

Again, examples of strong acids suitable for use in this reaction are methanesulfonic acid (MSA), mixtures of methanesulfonic acid and trifluoroacetic acid (TFA), e.g. an approx. 1:1 mixture of MSA and TFA, $ZnCl_2$. Methanesulfonic acid is a currently preferred choice. Alternative strong acids include H_2SO_4, $SnCl_4$, acetic acid, H_3PO_4, HF, BF_3 and BBr_3.

Typically, in the definitions of R1, R_2, R_3 and R_4, -O-C_{1-6}-alkyl is -O-C_{1-3}-alkyl, wherein -O-C_{1-3}-alkyl is preferably -OCH_3 or -OC_2H_5. Additionally, -S-C_{1-6}-alkyl may typically be -S-C_{1-3}-alkyl, wherein -S-C_{1-3}-alkyl may preferably be -SCH_3 or -SC_2H_5. -C_{1-6}-alkyl may be methyl, ethyl, p-propyl, isopropyl, p-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, hexyl or isohexyl. -C_{1-6}-alkyl may typically be -C_{1-3}-alkyl, wherein -C_{1-3}-alkyl may be methyl, ethyl or propyl (such as n-propyl or i-propyl). Preferably, R_2 and/or R_4 is hydroxyl, so that a 1,3-aromatic diol is included in compounds of formula **A**. Preferred compounds of formula A are those in which R_1 is halogen, preferably F or Cl. R_3 is preferably-O-C_{1-3}-alkyl, such as -OCH_3 or -OC_2H_5.

The term "-C_{2-6}-alkenyl" is intended to indicate a mono-, di-, or triunsaturated hydrocarbon radical comprising 2-6 carbon atoms, in particular 2-4 carbon atoms, such as 2-3 carbon atoms, e.g. vinyl, allyl, propenyl, butenyl, pentenyl or hexenyl.

The term "-C_{2-6}-alkynyl" is intended to indicate a hydrocarbon radical comprising 1-4 C-C triple bonds, e.g. 1, 2 or 3 triple bonds and 2-6 carbon atoms, the alkane chain typically comprising 2-5 carbon atoms, in particular 2-4 carbon atoms, such as 2-3 carbon atoms, e.g. ethynyl, propynyl, butynyl or pentynyl.

The term "heterocycloalkyl" is intended to include a cycloalkyl radical, wherein "cycloalkyl" indicates a saturated cycloalkane radical, comprising 3-8 carbon atoms, such as 4-7 or 3-6 carbon atoms, such as 4-6 or preferably 5-6 carbon atoms, e.g. cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl, said "heterocycloalkyl" comprising 1-7 carbon atoms, such as 1-6 carbon atoms, in particular a 4-, 5- or 6- membered ring, comprising 2-5 carbon atoms and 1-5 hetero atoms (selected from O, S and N), such as 3-5 carbon atoms and 1-3 hetero atoms, preferably 4-5 carbon atoms and 1-2 hetero atoms selected from O, S, or N, e.g. morpholino, morpholinyl, pyrrolidinyl, oxo-pyrrolidinyl, piperidino, azetidinyl, tetrahydro-furyl, tetrahydro-pyranyl, oxo-tetrahydro-furyl, oxo-oxazolidinyl, oxetanyl, dioxo-imidazolidinyl, piperidyl or piperazinyl. Preferred heterocycloalkyl radicals include pyrrolidinyl, piperazinyl and imidazolidinyl.

The term "heteroaryl" is intended to include radicals of (a) heterocyclic aromatic ring(s), comprising 1-4 heteroatoms (selected from O, S and N) and 1-10 carbon atoms, such as 1-3 heteroatoms and 1-6 carbon atoms, such as 1-3 heteroatoms and 2-5 carbon atoms, such as 1-2 heteroatoms and 3-5 carbon atoms, preferably 5- or 6- membered rings with 1-3 heteroatoms and 2-5 carbon atoms or 1-3 heteroatoms and 2-4 carbon atoms selected from O, S and N, e.g. pyridyl, thiazolyl, imidazolyl, isoxadiazolyl, [l,2,4]oxadiazolyl, oxazolyl, pyrazolyl, indolyl, thienyl, furyl, 1- benzo[b]thiophenyl, 2,3-dihydro-benzo[l,4]dioxinyl, or 2,3-dihydro-benzofuryl. Preferred heteroaryl radicals include pyridyl, 1,2,3-triazolyl and furyl.

The term "DOTA" stands for 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid.

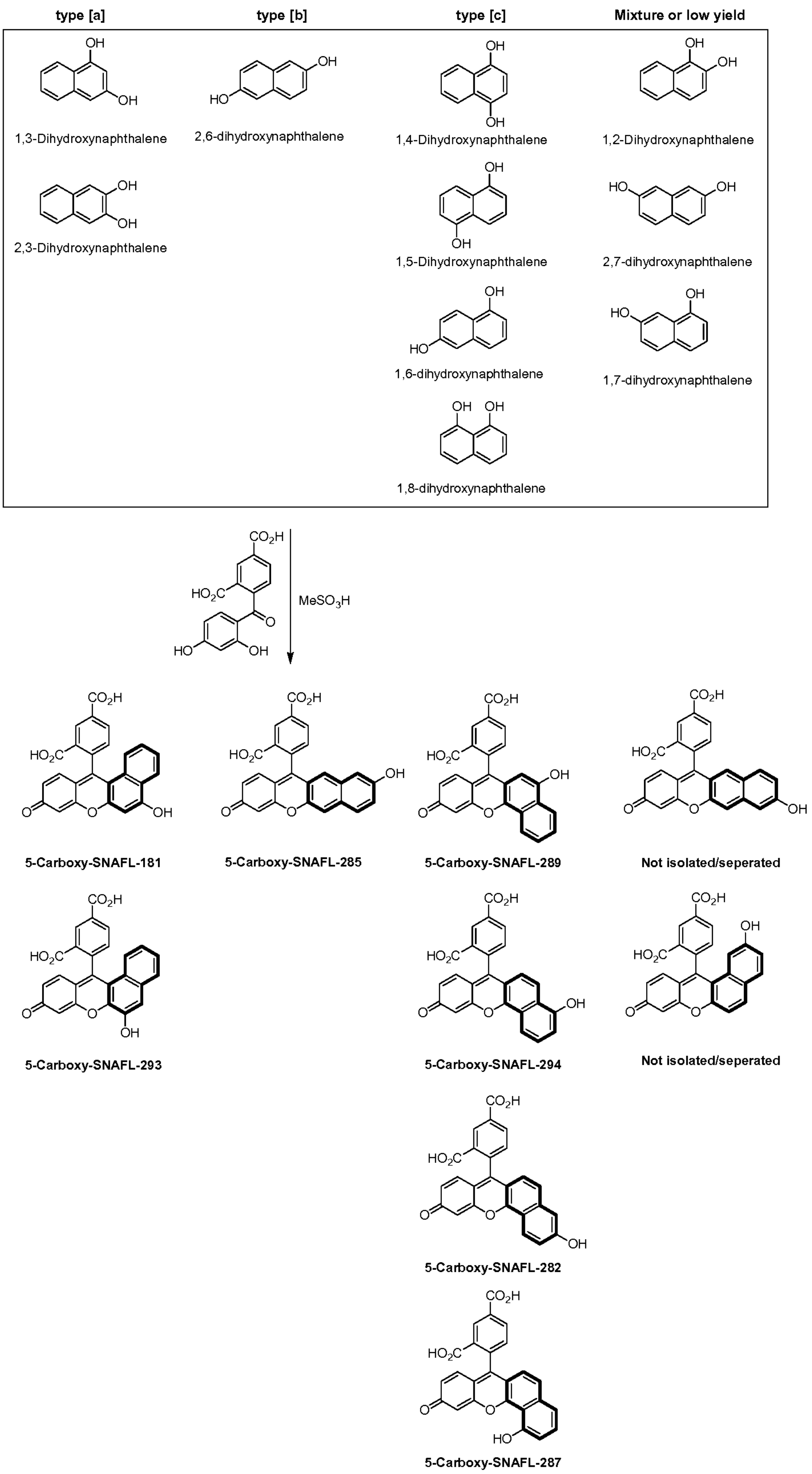
The term "biotin" stands for 5-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoic acid.

The term "maleimide" stands for 2,5-pyrroledione.

The term *"together with the intervening atoms may form an optionally substituted aromatic ring or ring system"* is intended to mean that an aromatic ring of an aromatic ring system is fused to the benzene ring to which the substituent pairs are attached. Examples of aromatic rings are a benzene ring and a pyridine ring.

Preferably, R_3/R_4 together with the intervening atoms may form an optionally substituted aromatic ring or ring system while R_2 is hydroxy. The compound of formula A may therefore be a dihydroxynaphthalene, as illustrated in Scheme 3.

Such aromatic rings or ring systems may (or may not) be substituted with one or more substituents selected from hydrogen; halogen; hydroxyl; nitro; cyano; mercapto; -O-C_{1-3}-alkyl; -S–C_{1-3}-alkyl; cyclopropyl; -C_{1-3}-alkyl; -C_{2-3}-alkenyl; or -C_{2-3}-alkynyl.


type [a]
type [b]
type [c]
Mixture or low yield
1,3-Dihydroxynaphthalene
2,6-dihydroxynaphthalene
1,4-Dihydroxynaphthalene
1,2-Dihydroxynaphthalene
2,3-Dihydroxynaphthalene
1,5-Dihydroxynaphthalene
2,7-dihydroxynaphthalene
1,6-dihydroxynaphthalene
1,7-dihydroxynaphthalene
1,8-dihydroxynaphthalene
MeSO3H
5-Carboxy-SNAFL-181
5-Carboxy-SNAFL-285
5-Carboxy-SNAFL-289
Not isolated/seperated
5-Carboxy-SNAFL-293
5-Carboxy-SNAFL-294
Not isolated/seperated
5-Carboxy-SNAFL-282
5-Carboxy-SNAFL-287

Scheme 3

The condensation reaction between the compound of formula **A** and compound **5** or compound **6** or compound **13**, respectively, is typically conducted for 10-40 hours at 50-100 °C, either with or without an inert atmosphere.

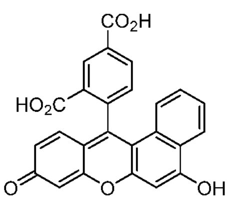
The condensation product mixture is typically worked up by quenching the reaction (e.g. by addition of water) and the sedimented product is isolated (e.g. by centrifuging, decantation or both). Further purification steps may include recrystallization, drying, washing and chromatographic separation, as required.

It should be understood that in the preparation of the carboxy-fluoresceins of the formula **B**, it is not a prerequisite that the compound **5** or compound **6** or compound **13** (as the case may be) are prepared according to the method described hereinabove. The method is equally applicable when using compound **5** or compound **6** or compound **13** obtained from other sources.

The choice of the compound of formula **A** will be decisive for the structure of the target compound of formula **B** and the target compound of formula **C**. For instance, as illustrated in Scheme 3, A may be a dihydroxynaphthalene, such as 1,3-dihydroxynaphthalene, 2,3-dihydroxynaphthalene, 2,6-dihydroxynaphthalene, 1,4-dihydroxynaphthalene, 1,5-dihydroxynaphthalene, 1,6-dihydroxynaphthalene, 1,8-dihydroxynaphthalene, 1,2-dihydroxynaphthalene, 2,7-dihydroxynaphthalene or 1,7-dihydroxynaphthalene.

An interesting compound **B** derived from compound **5** is 4-(6-hydroxy-3-oxo-3H-xanthen-9-yl)isophthalic acid (8).

Some of the most interesting compounds **B** derived from compound **6** are:



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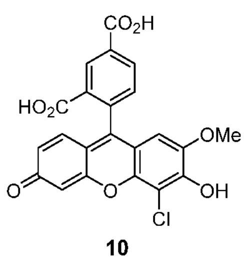

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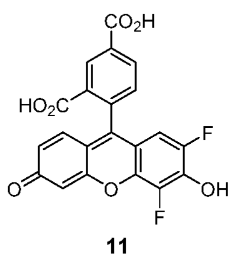

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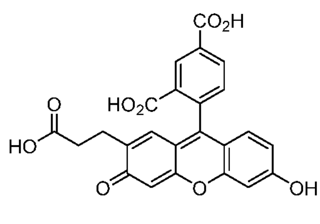
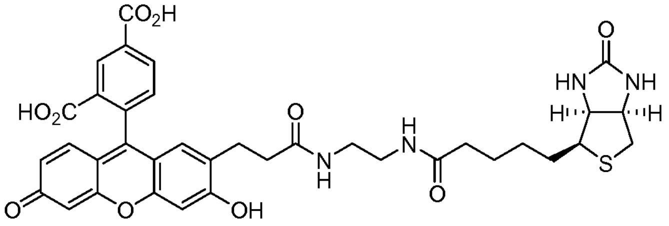
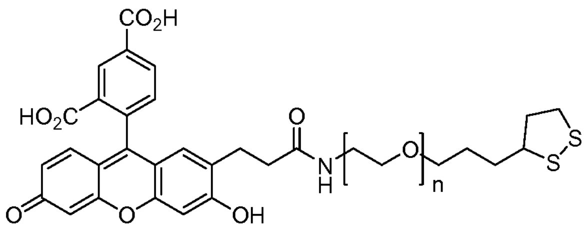


20

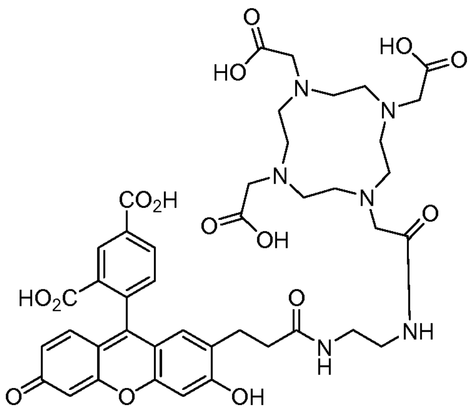
Other interesting compounds **B** derived from compound **6** are:



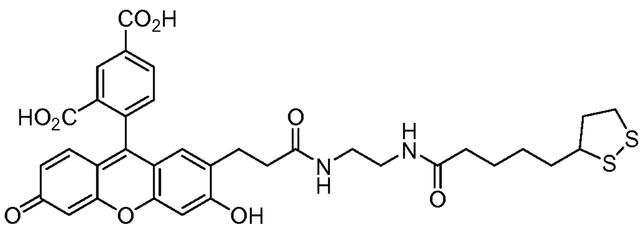
Biotin conjugate



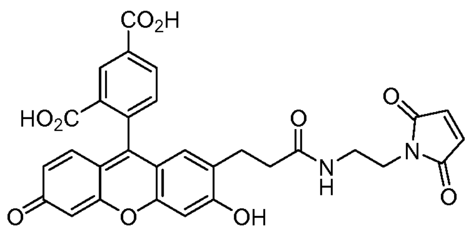
Gold PEG linker



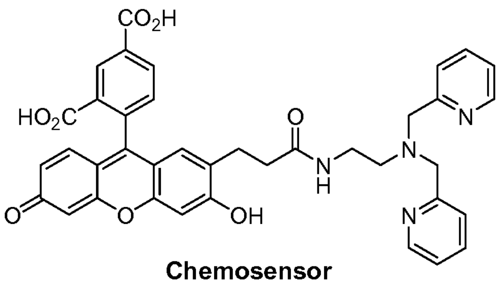
DOTA linker



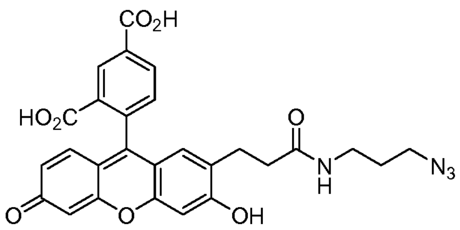
Gold linker



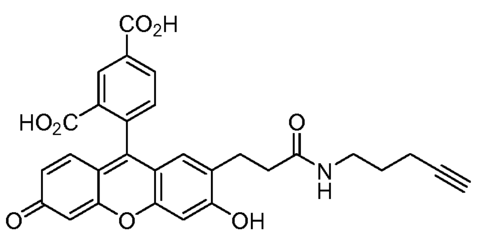
Maleimide linker



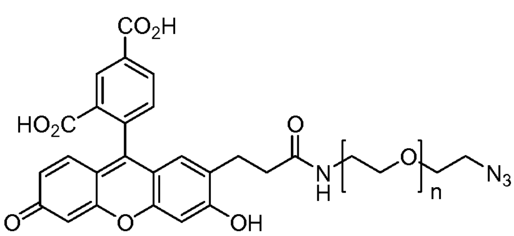
Chemosensor



Click-chemistry (Azide)

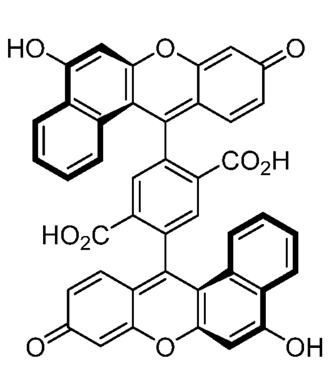


Click-chemistry (Alkyne)

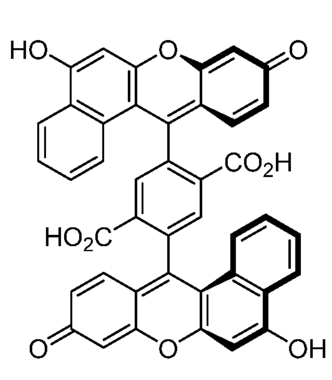


Click-chemistry (PEG-Azide)

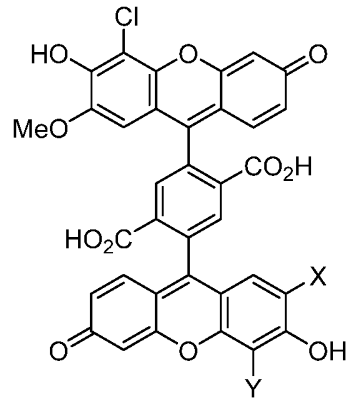
Some of the most interesting compounds **C** derived from compound **13** are:



16 *syn*



16 *anti*

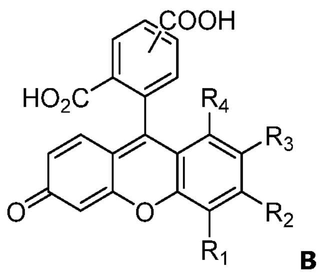


17 X = Y = H
18 X = OMe, Y = Cl

Novel carboxy-fluoresceins

It is believed that some of the carboxy-fluoresceins of formula **B** and of formula **C** which are obtainable from the method described further above represent hitherto unknown chemical entities.

Hence, the invention further provides novel compounds of formula **B***

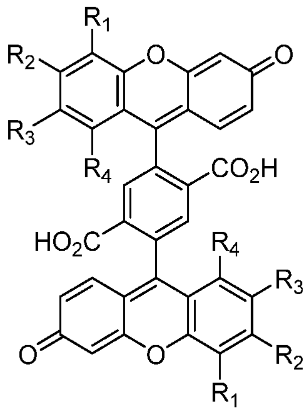


B*

wherein R_1, R_2, R_3 and R_4 are independently selected from hydrogen; halogen; hydroxyl; nitro; cyano; mercapto; -O-C_{1-3}-alkyl; -S–C_{1-3}-alkyl; cyclopropyl; -C_{1-3}-alkyl; -C_{2-3}-alkenyl; or -C_{2-3}-alkynyl; which -O-C_{1-3}-alkyl, -S–C_{1-3}-alkyl, cyclopropyl, -C_{1-3}-alkyl, -C_{2-3}-alkenyl or -C_{2-3}-alkynyl is optionally substituted with at least one substituent selected from halogen, hydroxyl, nitro, cyano and mercapto; with the additional option that any of the substituent pairs, R_1/R_2, R_2/R_3 and R_3/R_4 together with the intervening atoms may form an optionally substituted aromatic ring or ring system, with the proviso that when $R_1 = R_3 = R_4$ = hydrogen, then R_2 is different from hydroxyl.

Suitably, in compounds **B***, R_1, R_2, R_3 and R_4 are not all hydrogen. Preferably, in compounds B*, R_2 is hydroxyl (-OH). Suitably, R_1 is halogen, most preferably F or Cl. R_3 may be halogen, preferably F or Cl, or -O-C_{1-3}-alkyl, such as -OCH_3. Suitably, R_3/R_4 together with the intervening atoms form an optionally substituted aromatic ring system. Preferred compounds **B*** of the invention are compounds 9, 10 and 11 of Scheme 1.

Also, the invention further provides novel compounds of formula **C***

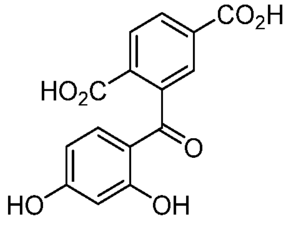


C*

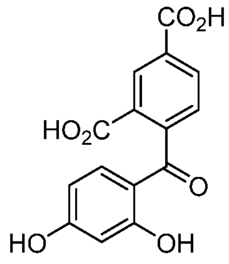
wherein R_1, R_2, R_3 and R_4 are independently selected from hydrogen; halogen; hydroxyl; nitro; cyano; mercapto; -O-C_{1-3}-alkyl; -S–C_{1-3}-alkyl; cyclopropyl; -C_{1-3}-alkyl; -C_{2-3}-alkenyl; or -C_{2-3}-alkynyl; which -O-C_{1-3}-alkyl, -S–C_{1-3}-alkyl, cyclopropyl, -C_{1-3}-alkyl, -C_{2-3}-alkenyl or -C_{2-3}-alkynyl is optionally substituted with at least one substituent selected from halogen, hydroxyl, nitro, cyano and mercapto; with the additional option that any of the substituent pairs, R_1/R_2, R_2/R_3 and R_3/R_4 together with the intervening atoms may form an optionally substituted aromatic ring or ring system, with the proviso that when R_1 = R_3 = R_4 = hydrogen, then R_2 is different from hydroxyl.

Suitably, in compounds **C***, R_1, R_2, R_3 and R_4 are not all hydrogen. Preferably, in compounds B*, R_2 is hydroxyl (-OH). Suitably, R_1 is halogen, most preferably F or Cl. R_3 may be halogen, preferably F or Cl, or -O-C_{1-3}-alkyl, such as -OCH_3. Suitably, R_3/R_4 together with the intervening atoms form an optionally substituted aromatic ring system. Preferred compounds **C*** of the invention are compounds 16 *syn*, 16 *anti*, 17 and 18 of Scheme 2.

Also, the invention further provides the novel compounds **5** and **6** of the formulae



5



6.

EXPERIMENTAL SECTION

Schemes 1 and 2

Unless otherwise stated, all starting materials were obtained from commercial suppliers and used as received. Solvents were HPLC grade and were used as received. High resolution mass spectra (HR-MS) were measured on a Ultimate 3000 Dionex UHPLC, Bruker Maxis 3G QTOF ESI MS. Reverse phase analytical LCMS was run on a Waters Acquity Ultra Performance LCMS. NMR spectra were recorded using a Varian Mercury 300 MHz spectrometer or a Bruker 500 MHz spectrometer. Chemical shifts were measured in ppm and coupling constants in Hz, the field is indicated in each case. When DMSO-d_6 was used, the values were δ 2.50 for ^{1}H NMR and δ 39.43 for ^{13}C NMR spectra. When D_2O added NaOD was used as solvent, the residual peak was used as internal reference at δ 4.79 for ^{1}H NMR spectrum. Melting points were measured with a Buch & Holm melting point apparatus and are uncorrected. TLC was performed on Merck aluminum sheets pre-coated with silica gel 60 F254. Gravity feed column chromatography was performed on Merck Kiselgel 60 (0.040 – 0.063 mm).

2-(2,4-Dihydroxybenzoyl)terephthalic acid (5) and 4-(2,4-dihydroxybenzoyl)isophthalic acid (6). In a 250 mL conical flask equipped with a reflux condenser and a magnetic stirrebar were placed 1,3-dioxo-1,3-dihydroisobenzofuran-5-carboxylic acid (20 g, 0.104 mol) and resorcinol (23 g, 0.208 mol) in 100 mL methanesulfonic acid. The reaction mixture were stirred at 80 °C overnight, added to 500 mL ice water under stirring and filtered. The solid residue was refluxed in 200 mL EtOH, added H_2O until precipitation, cooled to room temperature, filtered and dried *in vacuo* yielding 36.5 g (93 %) of crude 5(6)-Carboxy-fluorescein as an orange powder (only compound seen on LCMC). The crude compound was used without further purification. In a 500 mL conical flask equipped with a reflux condenser and a magnetic stirrebar were placed H_2O (200 g) and NaOH (200 g) were added under heat evolution. To the warm mixture were added crude 5(6)-Carboxy-fluorescein and the mixture was stirred at 80 °C overnight at which time the solution had become clear and almost colorless. The solution was added to 300 g ice and further cooled with ice. 12 M HCl were added slowly under stirring until a white compound precipitates (pH = 1-2). The mixture was left at 5 °C overnight, filtered and dried *in vacuo* yielding the crude mixture of isomers (approximately a 1:1 ratio) as an off white solid. The mixture was fractional crystallized by dissolving the mixture in MeOH (100 mL) and subsequently adding H_2O (3 L). Small crystals starts forming on the surface of the solution overnight and the solution is left standing at RT for one week in an open Erlenmeyer flask. The crystals are collected and the mother liquor is extracted with diethyl ether. The ether phase was evaporated to dryness, and crystallized using the same procedure as before in MeOH-H_2O. The combined solid (benzophenone **6**) was recrystallized 2-3 times, each time combining the mother liquor (containing mostly benzophenone **5**), yielding 11.5 g (36 %) of benzophenone **6**. Isolation of benzophenone **5** was achieved by combining the dried ether phases and crystallizing them in H_2O 4-5 times yielding 8.3 g (26 %).

2-(2,4-Dihydroxybenzoyl)terephthalic acid (5)

Mp: 271-274 (decompose); 1H NMR (400 MHz, DMSO) δ 13.54 (s, 2H), 12.01 (s, 1H), 10.75 (s, 1H), 8.16 (dd, *J* = 8.1, 1.7 Hz, 1H), 8.09 (d, *J* = 8.0 Hz, 1H), 7.85 (d, *J* = 1.4 Hz, 1H), 6.99 (d, *J* = 8.7 Hz, 1H), 6.34 – 6.29 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 199.41, 166.74, 166.48, 165.58, 164.72, 140.70, 135.16, 134.30, 133.81, 130.91, 130.84, 128.38, 113.61, 108.91, 103.02; MS (ESI$^+$) *m/z* [M + H$^+$] calcd for $C_{15}H_{11}O_7^+$ 303.0, found 302.9. HR-MS (ESI): *m/z* [M + H$^+$] calcd for $C_{15}H_{11}O_7^+$ 303.0499 found 303.0503.

4-(2,4-Dihydroxybenzoyl)isophthalic acid (6)

Mp: 265-267 (decompose); 1H NMR (500 MHz, DMSO) δ 13.48 (s, 1H), 12.00 (s, 1H), 10.76 (s, 1H), 8.51 (d, *J* = 1.7 Hz, 1H), 8.23 (dd, J = 7.9, 1.7 Hz, 1H), 7.56 (d, J = 7.9 Hz, 1H), 6.96 (d, J = 8.8 Hz, 1H), 6.45 – 6.17 (m, 1H); ^{13}C NMR (126 MHz, DMSO) δ 199.20, 166.03, 165.91, 165.12, 164.13, 143.83, 134.58, 132.84, 131.79, 130.68, 129.72, 127.95, 113.10, 108.43, 102.49. MS (ESI$^+$) *m/z* [M + H$^+$] calcd for $C_{15}H_{11}O_7^+$ 303.0, found 302.9. HR-MS (ESI): *m/z* [M + H$^+$] calcd for $C_{15}H_{11}O_7^+$ 303.0499 found 303.0503.

General Method. 2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)terephthalic acid (7). In a 20 mL conical flask were placed 2-(2,4-dihydroxybenzoyl)terephthalic acid (**5**) (200 mg, 0.66 mmol), resorcinol (80 mg, 0.72 mmol) in 5 mL methanesulfonic acid. The reaction mixture were stirred at RT overnight, added to 50 mL ice water under stirring and filtered. The solid residue was dissolved in 2M NaOH (40 mL), precipitated with 2M HCl and filtered. The crude compound was re-precipitated first in EtOH/H_2O and followed by NaOH/HCl, filtered and dried *in vacuo.* Yield: 245 mg, 98 %; Mp: >300 °C; ^{1}H NMR (400 MHz, D_2O(NaOD)) δ 8.04 (dd, *J*

= 8.0, 1.7 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 1.3 Hz, 1H), 7.21 – 7.13 (m, 2H), 6.64 – 6.54 (m, 4H); ^{13}C NMR (101 MHz, D_2O(NaOD)) δ 180.61, 174.86, 174.35, 158.85, 158.65, 141.76, 137.05, 131.55, 131.30, 130.24, 129.72, 128.10, 122.87, 112.50, 103.52; MS (ESI$^+$) m/z [M + H$^+$] calcd for $C_{21}H_{13}O_7^+$ 377.1, found 377.0; HR-MS (ESI): m/z [M + H$^+$] calcd for $C_{21}H_{13}O_7^+$ 377.0655 found 377.0676.

4-(6-hydroxy-3-oxo-3H-xanthen-9-yl)isophthalic acid (8). The compound was prepared as in the case of compound **7**, starting from 4-(2,4-Dihydroxybenzoyl)isophthalic acid (**6**) (0.5 g, 1.65 mmol) and resorcinol (0.2 g, 1.82 mmol) and 10 mL methanesulfonic acid. Yield: 602 mg, 96 %; Mp: >300 °C; ^{1}H NMR (400 MHz, D_2O(NaOD)) δ 8.21 (d, J = 1.6 Hz, 1H), 8.00 (dd, J = 7.9, 1.7 Hz, 1H), 7.24 (d, J = 7.9 Hz, 1H), 7.13 (d, J = 9.2 Hz, 2H), 6.58 (dd, J = 9.2 Hz, J = 2.2 Hz, 2H), 6.54 (d, J = 2.2 Hz, 2H); ^{13}C NMR (101 MHz, D_2O(NaOD))) δ 180.59, 174.74, 174.49, 158.67, 139.59, 137.36, 134.08, 131.40, 130.05, 129.45, 128.43, 122.88, 112.19, 103.58; MS (ESI$^+$) m/z [M + H$^+$] calcd for $C_{21}H_{13}O_7^+$ 377.1, found 377.1; HR-MS (ESI): m/z [M + H$^+$] calcd for $C_{21}H_{13}O_7^+$ 377.0655 found 377.0667.

4-(5-hydroxy-9-oxo-9H-benzo[a]xanthen-12-yl)isophthalic acid (9) The compound was prepared as in the case of compound **7**, starting from 4-(2,4-dihydroxybenzoyl)isophthalic acid (**6**) (500 mg, 1.65 mmol), naphthalene-1,3-diol (500 mg, 3.12 mmol) and 10 mL methanesulfonic acid. Yield: 557 mg, 79 %; Mp: >300 °C; ^{1}H NMR (400 MHz, D_2O(NaOD)) δ 8.22 (d, J = 1.5 Hz, 1H), 8.10 (dd, J = 8.1, 1.3 Hz, 1H), 7.78 (dd, J = 7.9, 1.8 Hz, 1H), 7.37 – 7.28 (m, 1H), 7.08 (ddd, J = 8.6, 7.1, 1.5 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.81 – 6.74 (m, 1H), 6.71 (d, J = 7.9 Hz, 1H), 6.47 (dd, J = 7.5, 2.2 Hz, 2H), 6.33 (s, 1H); ^{13}C NMR (101 MHz, D_2O(NaOD)) δ 180.42, 176.93, 174.50, 173.99, 162.98, 155.72, 155.05, 138.58, 138.12, 137.14, 131.55, 130.83, 130.25, 129.84, 129.51, 129.43, 128.57, 126.71, 126.26, 124.66, 121.20, 111.73, 109.80, 103.14, 101.30; MS (ESI$^+$) m/z [M + H$^+$] calcd for $C_{25}H_{15}O_7^+$ 427.1 found 427.1; HR-MS (ESI): m/z [M + H$^+$] calcd for $C_{25}H_{15}O_7^+$ 427.0812 found 427.0835.

4-(5-chloro-6-hydroxy-7-methoxy-3-oxo-3H-xanthen-9-yl)isophthalic acid (10) The compound was prepared as in the case of compound **7**, starting from 4-(2,4-dihydroxybenzoyl)isophthalic acid (**6**) (300 mg, 0.99 mmol), 2-chloro-4-methoxybenzene-1,3-diol (200 mg, 1.15 mmol) and 10 mL methanesulfonic acid. The crude compound was purification by silica gel dry column vacuum chromatography was performed by dissolving the crude compound in MeOH and 2 drops of 12 M NaOH(aq), evaporation on celite in vacuo, using 2 % AcOH in CH_2Cl_2/MeOH with 5 % increments. The compound was re-precipitated in NaOH/HCl, filtered and dried *in vacuo.* Yield: 265 mg, 60 %; Mp: >300 °C; ^{1}H NMR (400 MHz, D_2O(NaOD)) δ 8.21 (d, J = 1.6 Hz, 1H), 8.04 (dd, J = 7.9, 1.7 Hz, 1H), 7.27 (d, J = 7.9 Hz, 1H), 7.05 (d, J = 9.2 Hz, 1H), 6.65 (d, J = 2.2 Hz, 1H), 6.59 (dd, J = 9.2, 2.2 Hz, 1H), 6.31 (s, 1H), 3.57 (s, 3H); ^{13}C NMR (101 MHz, D_2O(NaOD)) δ 178.91, 174.64, 174.41, 168.26, 157.27, 156.08, 150.98, 150.93, 139.71, 137.41, 134.05, 130.38, 130.27, 129.54, 128.57, 122.81, 111.86, 110.88, 107.82, 103.60, 55.34; MS (ESI$^+$) *m/z* [M + H$^+$] calcd for $C_{22}H_{14}ClO_8^+$ 441.0, found 441.0; HR-MS (ESI): *m/z* [M + H$^+$] calcd for $C_{22}H_{14}ClO_8^+$ 441.0372 found 441.0377.

4-(5,7-difluoro-6-hydroxy-3-oxo-3H-xanthen-9-yl)isophthalic acid (11) The compound was prepared as in the case of compound **7**, starting from 4-(2,4-dihydroxybenzoyl)isophthalic acid (**6**) (600 mg, 1.98 mmol), 2,4-difluorobenzene-1,3-diol (440 mg, 3.9 mmol) and 10 mL methanesulfonic acid. The crude compound was purification by silica gel dry column vacuum chromatography was performed by dissolving the crude compound in MeOH and 2 drops of 12 M NaOH(aq), evaporation on celite in vacuo, using 2 % AcOH in CH_2Cl_2/MeOH with 5 % increments. The compound was re-precipitated in NaOH/HCl, filtered and dried *in vacuo.* Yield: 237 mg, 28 %; Mp = 259-265 °C (decompose); ^{1}H NMR (400 MHz, D_2O(NaOD)) δ 8.16 (d, *J* = 1.4 Hz, 1H), 7.97 (d, *J* = 7.9 Hz, 1H), 7.25 (d, *J* = 7.9 Hz, 1H), 7.09 (d, *J* = 9.4 Hz, 1H), 6.74 (dd, *J* = 11.6, 1.3 Hz, 1H), 6.56 – 6.49 (m, 2H); ^{13}C NMR (101 MHz, D_2O(NaOD)) δ 182.02, 174.44, 174.43, 158.49, 139.54, 137.67, 133.65, 131.55, 130.02, 129.64, 128.65, 124.10, 113.14, 108.53, 108.32, 103.71; ^{19}F NMR (282 MHz, D_2O(NaOD)) δ 127, 154; MS (ESI$^+$) *m/z* [M + H$^+$] calcd for $C_{21}H_{11}F_2O_7^+$ 413.0, found 413.0; HR-MS (ESI): *m/z* [M + H$^+$] calcd for $C_{21}H_{11}F_2O_7^+$ 413.0467 found 413.0478.

2,5-bis(2,4-dihydroxybenzoyl)terephthalic acid (13). In a 100 mL conical flask equipped with a reflux condenser and a magnetic stirrebar were placed benzo[1,2-c:4,5-c']difuran-1,3,5,7-tetraone (**12**) (5 g, 22.9 mmol) and resorcinol (10 g, 91.6 mmol) in 60 mL methanesulfonic acid. The reaction mixture were stirred at 80 °C overnight, added to 500 mL ice water under stirring and filtered. The solid residue was refluxed in 200 mL EtOH, cooled to room temperature, filtered and dried *in vacuo* yielding 12 g of crude 2,5-bis(3,6-dihydroxy-9-(methoxysulfonyl)-9H-xanthen-9-yl)terephthalic acid as an orange powder. The crude compound was used without further purification. In a 250 mL conical flask equipped with a reflux condenser and a magnetic stirrebar were placed H_2O (100 g) and NaOH (100 g) were added under heat evolution. To the warm mixture were added crude 2,5-bis(3,6-dihydroxy-9-(methoxysulfonyl)-9H-xanthen-9-yl)terephthalic acid and the mixture were stirred at 80 °C overnight at which time the solution had become clear and almost colorless. The solution was added to 200 g ice and further cooled with ice. 12M HCL were added slowly under stirring until a white compound precipitates (pH = 1-2). The mixture was left overnight at 5 °C, filtered and the solid was dried *in vacuo* yielding a white powder. Yield: 5.5 g, 55 %; Mp: 283-286 (decompose); ^{1}H NMR (500 MHz, DMSO) δ 13.70 (s, 2H), 11.92 (s, 2H), 10.91 (s, 2H), 7.90 (s, 2H), 7.17 (d, *J* = 8.7 Hz, 2H), 6.49 – 6.15 (m, 4H); ^{13}C NMR (101 MHz, DMSO)) δ 197.95, 165.61, 165.34, 164.12, 140.97, 134.82, 132.62, 128.79, 113.12, 108.55, 102.54; MS (ESI^+) m/z [$M + H^+$] calcd for $C_{25}H_{15}O_{10}^+$ 439.1, found 439.0; HR-MS (ESI): m/z [$M + H^+$] calcd for $C_{25}H_{15}O_{10}^+$ 439.0659 found 439.0658.

General Method. Mixture of 2-(5,7-difluoro-6-hydroxy-3-oxo-3H-xanthen-9-yl)-5-(6-hydroxy-3-oxo-3H-xanthen-9-yl)terephthalic acid (14) and 2,5-bis(5,7-difluoro-6-hydroxy-3-oxo-3H-xanthen-9-yl)terephthalic acid (15). A mixture of 2,5-bis(2,4-dihydroxybenzoyl)terephthalic acid (**13**) (100 mg, 0.23 mmol) and 2,4-difluorobenzene-1,3-diol (100 mg, 0.7 mmol) in methanesulfonic acid (20 mL) was placed in a 50 mL conical flask equipped with a magnetic stirrebar and the mixture was heated to 50 °C overnight. 2,4-difluorobenzene-1,3-diol (100 mg, 0.7 mmol) was added and the reaction was stirred for 2 days at 50 °C, added to 50 mL ice water under stirring and filtered. The solid residue was dissolved in 2M NaOH (40 mL), precipitated with 2M HCl and filtered. The crude compound was purified by dry column vacuum chromatography (5 % AcOH in Toluene to 40 % EtOH in 5 % AcOH in Toluene with 4 % increments) giving a mixture of compound **14** ((ESI^+) m/z [$M + H^+$] calcd for $C_{34}H_{17}F_2O_{10}^+$ 623.1 found 623.0) and compound **15** ((ESI^+) m/z [$M + H^+$] calcd for $C_{34}H_{15}F_4O_{10}^+$ 659.1 found 659.1

2,5-bis(5-hydroxy-9-oxo-9H-benzo[a]xanthen-12-yl)terephthalic acid (16 *anti*) and (16 *syn*) The compounds were prepared as in the case of compound **14**, starting from 2,5-bis(2,4-dihydroxybenzoyl)terephthalic acid (**13**) (500 mg, 1.14 mmol), naphthalene-1,3-diol (500 mg, 3.12 mmol) and 10 mL methanesulfonic acid. The reaction mixture was stirred at 80 °C for 2 hours. Yield: 665 mg, 85 %; Mp: >300 °C; ^{13}C NMR (101 MHz, $D_2O(NaOD)$) δ 13C NMR (101 MHz, D2O) δ 180.79, 180.65, 177.12, 176.96, 172.71, 172.64, 163.54, 163.48, 156.09, 156.01, 154.54, 154.39, 140.74, 140.70, 137.34, 137.23, 131.87, 131.81, 130.63, 130.60, 130.07, 129.85, 129.50, 129.40, 129.36, 127.19, 127.01, 126.54, 126.48, 124.97, 124.92, 121.58, 121.53, 111.94, 111.71, 110.31, 110.27, 103.29, 103.22, 101.38, 101.35, 99.99.; MS (ESI^+) m/z [$M + H^+$] calcd for $C_{42}H_{23}O_{10}^+$ 687.1 found 687.1. HR-MS (ESI-TOF): m/z calcd for $C_{42}H_{23}O_{10}^+$ 687.1286 found 687.1289.

2-(5-chloro-6-hydroxy-7-methoxy-3-oxo-3H-xanthen-9-yl)-5-(6-hydroxy-3-oxo-3H-xanthen-9-yl)terephthalic acid (17) and 2,5-bis(5-chloro-6-hydroxy-7-methoxy-3-oxo-3H-xanthen-9-yl)terephthalic acid (18) The compounds were prepared as in the case of compound **18**, starting from 2,5-bis(2,4-dihydroxybenzoyl)terephthalic acid (**14**) (100 mg, 0.23 mmol), 2-chloro-4-methoxybenzene-1,3-diol (90 mg, 0.52 mmol) and 3 mL methanesulfonic acid. **Compound 17.** Yield: 34 mg, 23 %; Mp: >300 °C; ^1H NMR (400 MHz, $D_2O(NaOD)$) δ 8.04 (s, 1H), 7.83 (s, 1H), 7.35 (dd, J = 19.6, 9.2 Hz, 2H), 7.26 (d, J = 9.2 Hz, 1H), 6.80 (d, J = 2.2 Hz, 1H), 6.72 – 6.70 (m, 1H), 6.70 – 6.68 (m, 2H), 6.66 (d, J = 2.3 Hz, 1H), 6.65 – 6.64 (m, 2H), 6.54 (s, 1H), 3.74 (s, 3H); ^{13}C NMR (101 MHz, $D_2O(NaOD)$) δ 180.75, 179.11, 173.68, 173.51, 168.39, 158.90, 158.86, 157.84, 157.52, 155.45, 151.12, 151.08, 140.55, 140.25, 132.90, 132.80, 131.44, 130.51, 130.33, 130.09, 123.18, 123.13, 122.99, 112.45, 112.42, 112.07, 111.26, 107.90, 103.95, 103.67, 103.63, 55.57; MS (ESI$^+$) *m/z* [M + H$^+$] calcd for $C_{35}H_{20}ClO_{11}^+$ 651.9 found 651.0. **Compound 18.** Yield: 34 mg, 21 %; Mp: >300 °C; ^{1}H NMR (400 MHz, D_2O(NaOD)) δ 7.96 (s, 1H), 7.34 (d, *J* = 9.2 Hz, 1H), 6.84 (d, *J* = 2.2 Hz, 1H), 6.72 (dd, *J* = 9.2, 2.2 Hz, 1H), 6.57 (s, 1H), 3.70 (s, 3H); ^{13}C NMR (101 MHz, D_2O(NaOD)) δ 179.10, 179.05, 173.58, 173.46, 168.33, 168.27, 157.46, 157.43, 155.43, 155.37, 151.03, 150.91, 140.55, 140.52, 132.90, 132.75, 130.53, 122.91, 111.97, 111.92, 111.20, 107.83, 107.78, 103.70, 55.54, 55.00; MS (ESI$^+$) *m/z* [M + H$^+$] calcd for $C_{36}H_{21}Cl_2O_{12}^+$ 716.4 found 715.0.

Scheme 3

Unless otherwise stated, all starting materials were obtained from commercial suppliers and used as received. Solvents were HPLC grade and were used as received. High resolution mass spectra (HR-MS) were measured on a Ultimate 3000 Dionex UHPLC, Bruker Maxis 3G QTOF ESI MS. Reverse phase analytical LCMS was run on a Water UPLC-MS. NMR spectra were recorded using a Varian Mercury 300 MHz spectrometer or a Bruker 500 MHz spectrometer. Chemical shifts were measured in ppm and coupling constants in Hz, the field is indicated in each case. When DMSO-d6 was used, the values were δ 2.50 for 1H NMR and δ 39.43 for 13C NMR spectra. When D2O added NaOD was used as solvent, the residual peak was used as internal reference at δ 4.79 for 1H NMR spectrum. Melting points were measured with a Buch & Holm melting point apparatus and are uncorrected. TLC was performed on Merck aluminum sheets pre-coated with silica gel 60 F254. Gravity feed column chromatography was performed on Merck Kieselgel 60 (0.040 – 0.063 mm).

General procedure for the syntheses of 5-carboxy-SNAFLs. 4-(2,4-Dihydroxybenzoyl)isophthalic acid (500 mg, 1.65 mmol) and the appropriate dihydroxynaphthalene (275 mg, 1.75 mmol) were dissolved in TFA (5 mL) and methanesulfonic acid (5 mL). The reaction mixture was stirred at room temperature overnight. The reaction was quenched by adding H_2O (25 mL) and the resulting dark purple precipitate was collected by centrifugation. After decantation the sediment was dissolved in NaOH(aq) (2 M, 15 mL) and precipitated with HCl(aq) (2 M, 20 mL). After decantation the sediment was washed with H_2O (2 x 35 mL) and re-precipitated by dissolving in EtOH (10 mL) and precipitated with H_2O (ad H_2O until precipitation). After decantation and washing with H_2O (2 x 35 mL) the crude compound was dried *in vacuo* yielding a dark purple powder. Further purification by silica gel dry column vacuum chromatography was performed by dissolving the crude compound in MeOH and 2 drops of 12 M NaOH(aq), evaporation on celite *in vacuo*, using 2 % AcOH in CH_2Cl_2/MeOH with 5 % increments was done if required.

5-Carboxy-SNAFL-282. Starting from 1,6-dihydroxynaphthalene (275 mg, 1.75 mmol) and 4-(2,4-dihydroxybenzoyl)isophthalic acid (500 mg, 1.65 mmol). Crude yield: 574 mg, 79 %; Mp: 267-271 °C; ^{1}H NMR (400 MHz, acetone-d6) δ 8.58 (dd, *J* = 1.4, 0.6 Hz, 1H), 8.48 (d, *J* = 9.1 Hz, 1H), 8.41 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.46 (dd, *J* = 8.0, 0.6 Hz, 1H), 7.41 (d, *J* = 8.7 Hz, 1H), 7.34 (dd, *J* = 9.1, 2.4 Hz, 1H), 7.26 (d, *J* = 2.4 Hz, 1H), 7.04 (d, *J* = 2.4 Hz, 1H), 6.82 (d, *J* = 8.7 Hz, 1H), 6.78 (d, *J* = 8.8 Hz, 1H), 6.72 (dd, *J* = 8.8, 2.4 Hz, 1H); ^{13}C NMR (101 MHz, acetone-d6) δ 169.68, 167.35, 161.33, 159.07, 158.97, 153.82, 148.94, 138.33, 137.94, 134.74, 131.26, 129.17, 127.73, 126.41, 126.08, 125.66, 124.09, 120.51, 119.82, 114.75, 111.75, 111.46, 111.25, 104.48, 85.13; MS (ESI$^+$) m/z [M + H$^+$] calcd for $C_{25}H_{14}O_7^+$ 427.1, found 427.1. HR-MS (ESI): m/z [M + H$^+$] calcd for $C_{25}H_{14}O_7^+$ 427.0812 found 427.0835.

5-Carboxy-SNAFL-285. Starting from 2,6-dihydroxynaphthalene (275 mg, 1.75 mmol) and 4-(2,4-dihydroxybenzoyl)isophthalic acid (500 mg, 1.65 mmol). Crude yield: 580 mg, 80 %; Mp: >300 °C; ^{1}H NMR (400 MHz, acetone-d6) δ 9.03 (s, 1/2H), 8.67 (s, 1H), 8.64 (s, 1/2H), 8.33 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.93 (d, *J* = 9.0 Hz, 1H), 7.46 (d, *J* = 9.0 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 2.7 Hz, 1H), 7.03 (d, *J* = 9.3 Hz, 1H), 6.91 (dd, *J* = 9.3, 2.7 Hz, 1H), 6.78 (d, *J* = 1.9 Hz, 1H), 6.67 (d, *J* = 2.3 Hz, 2H); ^{13}C NMR (101 MHz, acetone-d6) δ 170.16, 167.09, 160.88, 160.83, 155.78, 152.18, 150.69, 138.30, 135.14, 134.19, 133.46, 130.54, 129.16, 128.13, 126.86, 126.82, 125.77, 120.81, 120.26, 114.70, 113.19, 112.48, 109.95, 103.71, 85.27.; MS (ESI$^+$) m/z [M + H$^+$] calcd for $C_{25}H_{14}O_7^+$ 427.1, found 427.1. HR-MS (ESI): m/z [M + H$^+$] calcd for $C_{25}H_{14}O_7^+$ 427.0812 found 427.0833.

5-Carboxy-SNAFL-287. Starting from 1,8-dihydroxynaphthalene (50 mg, 312 µmol) and 4-(2,4-dihydroxybenzoyl)isophthalic acid (100 mg, 331 µmol). Purification by chromatography necessary. Yield: 78 mg, 59 %; Mp: 235-239 °C; ^{1}H NMR (400 MHz, acetone-d6) δ 8.59 (dd, *J* = 1.5, 0.7 Hz, 1H), 8.42 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.61 – 7.47 (m, 3H), 7.43 (dd, *J* = 8.1, 0.7 Hz, 1H), 7.15 (d, *J* = 2.4 Hz, 1H), 7.08 (dd, *J* = 7.7, 1.1 Hz, 1H), 6.85 (m, 2H), 6.78 (dd, *J* = 8.7, 2.4 Hz, 1H); ^{13}C NMR (101 MHz, acetone-d6) δ 169.56, 167.30, 161.40, 158.75, 156.13, 152.76, 149.97, 138.68, 138.07, 134.89, 131.14, 130.97, 128.95, 127.85, 126.45, 126.28, 125.68, 121.23, 115.45, 115.18, 114.41, 113.80, 111.36, 104.70, 84.32; MS (ESI$^+$) m/z [M + H$^+$] calcd for $C_{25}H_{14}O_7^+$ 427.1, found 427.1. HR-MS (ESI): m/z [M + H$^+$] calcd for $C_{25}H_{14}O_7^+$ 427.0812 found 427.0832.

5-Carboxy-SNAFL-289. Starting from 1,4-dihydroxynaphthalene (275 mg, 1.75 mmol) and 4-(2,4-dihydroxybenzoyl)isophthalic acid (500 mg, 1.65 mmol). Crude yield: 525 mg, 74 %; Mp: 268-272 °C; ^{1}H NMR (400 MHz, acetone-d6) δ 8.58 (s, 1H), 8.55 (d, *J* = 8.0 Hz, 1H), 8.42 (dd, *J* = 8.0, 1.3 Hz, 1H), 8.25 (d, *J* = 7.8 Hz, 1H), 7.75 (ddd, *J* = 8.2, 7.0, 1.3 Hz, 1H), 7.68 (ddd, *J* = 8.2, 7.0, 1.3 Hz, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.03 (d, *J* = 2.4 Hz, 1H), 6.84

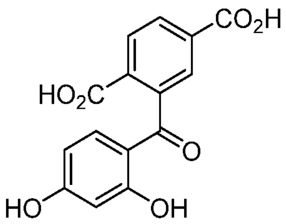
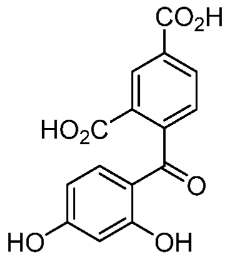
(d, *J* = 8.7 Hz, 1H), 6.71 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.15 (s, 1H); ^{13}C NMR (101 MHz, acetone-d6) δ 169.69, 167.45, 161.30, 158.91, 154.02, 150.78, 142.18, 137.99, 135.06, 131.27, 129.08, 128.98, 128.89, 128.05, 127.57, 126.59, 126.47, 124.13, 123.61, 114.56, 114.05, 111.04, 105.36, 104.37, 85.30.; MS (ESI$^+$) m/z [M + H$^+$] calcd for $C_{25}H_{14}O_7^+$ 427.1, found 427.1. HR-MS (ESI): m/z [M + H$^+$] calcd for $C_{25}H_{14}O_7^+$ 427.0812 found 427.0835.

5-Carboxy-SNAFL-293. Starting from 2,3-dihydroxynaphthalene (54 mg, 331 µmol) and 4-(2,4-dihydroxybenzoyl)isophthalic acid (100 mg, 331 µmol). Purification by chromatography necessary. Yield: 41 mg, 29 %; Mp: 205-208 °C; ^{1}H NMR (400 MHz, Acetone) δ 9.15 (s, 1H), 9.10 (s, 1H), 8.68 (d, *J* = 0.7 Hz, 5H), 8.34 (dd, J = 8.0, 1.5 Hz, 1H), 7.76 (d, *J* = 8.1 Hz, 1H), 7.52 (s, 6H), 7.42 (dd, *J* = 8.0, 0.6 Hz, 1H), 7.28 (ddd, *J* = 8.0, 5.9, 2.0 Hz, 6H), 7.11 – 6.98 (m, 2H), 6.97 – 6.91 (m, 1H), 6.71 (s, 1H), 6.70 (s, 2H); ^{13}C NMR (100 MHz, Acetone) δ 9.15, 9.10, 8.68, 8.68, 8.68, 8.68, 8.35, 8.34, 8.33, 8.32, 7.77, 7.75, 7.52, 7.43, 7.43, 7.41, 7.41, 7.30, 7.30, 7.29, 7.28, 7.28, 7.27, 7.26, 7.09, 7.08, 7.07, 7.06, 7.05, 7.05, 7.03, 6.95, 6.94, 6.94, 6.71, 6.70; MS (ESI$^+$) m/z [M + H$^+$] calcd for $C_{25}H_{14}O_7^+$ 427.4, found 427.1. HR-MS (ESI): m/z [M + H$^+$] calcd for $C_{25}H_{14}O_7^+$ 427.0812 found 427.0825.

5-Carboxy-SNAFL-294. Starting from 1,5-dihydroxynaphthalene (54 mg, 331 µmol) and 4-(2,4-dihydroxybenzoyl)isophthalic acid (100 mg, 331 µmol). Crude yield: 105 mg, 75 %; Mp: 243-245 °C; ^{1}H NMR (400 MHz, acetone-d6) δ 8.61 (dd, *J* = 1.5, 0.6 Hz, 1H), 8.42 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.94 (dd, *J* = 8.9, 0.6 Hz, 1H), 7.59 – 7.51 (m, 1H), 7.49 (dd, *J* = 8.0, 0.6 Hz, 1H), 7.12 (dd, *J* = 7.6, 0.7 Hz, 1H), 7.07 (d, *J* = 2.4 Hz, 1H), 6.87 (d, *J* = 8.7 Hz, 1H), 6.85 (d, *J* = 8.9 Hz, 1H), 6.75 (dd, *J* = 8.7, 2.4 Hz, 1H); ^{13}C NMR (101 MHz, acetone-d6) δ 168.70, 166.27, 160.60, 157.99, 154.09, 152.97, 147.65, 137.06, 133.53, 130.36, 128.40, 128.19, 126.96, 126.45, 126.04, 125.63, 123.18, 118.92, 114.03, 113.93, 113.57, 111.46, 110.72, 103.59, 39.70; MS (ESI$^+$) m/z [M + H$^+$] calcd for $C_{25}H_{14}O_7^+$ 427.1, found 427.1. HR-MS (ESI): m/z [M + H$^+$] calcd for $C_{25}H_{14}O_7^+$ 427.0812 found 427.0833.

The following are aspects of the invention

Aspect 1. A method for the preparation and isolating of compound **6** and, optionally, of compound **5**

 **5**  **6**

said method comprising the steps of:

(i) providing a condensation product mixture, being the result of a condensation reaction between trimellitic anhydride and resorcinol mediated by acid;

(ii) hydrolysing said condensation product mixture with a strong aqueous base at pH at least 11;

(iii) acidifying the reaction mixture of step (ii) so as to isolate a mixture of compound **5** and compound **6**;

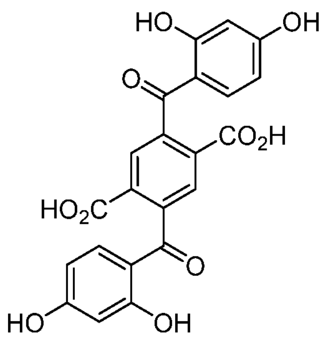
(iv) dissolving the mixture of compound **5** and compound **6** in methanol and adding water so as to precipitate compound **6**;

(v) extracting the mother liquor with an organic solvent so as to isolate compound **5** and any remaining compound **6**, and removing the organic solvent so as to obtain a dried extract;

(vi) optionally repeating steps (iv) and (v) in one or more additional cycles using the dried extract obtained in step (v);

(vii) optionally dissolving the dried extract obtained in step (v) in refluxing H_2O and precipitating compound **5**.

Aspect 2. A method for the preparation and isolation of compound **13**



13

said method comprising the steps of:

(i) providing a condensation product, being the result of a condensation reaction between pyromellitic dianhydride and resorcinol mediated by acid;

(ii) hydrolysing said condensation product with a strong aqueous base at pH of at least 11;

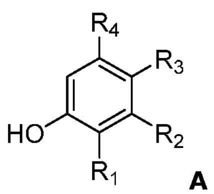
(iii) acidifying the reaction mixture of step (ii) so as to isolate compound **13**.

Aspect 3. The method according to any one of aspects 1-2, wherein hydrolysis steps (step ii.) are carried out at a pH of 12-14, preferably using a 1:1 weight ratio mixture of NaOH and H_2O.

Aspect 4. The method according to any one of aspects 1-3, wherein the acidification steps (step iii) are carried out using 12 M HCl.

Aspect 5. The method according to any one of aspects 1, 3 or 4, wherein, in step vi, steps (iv) and (v) are repeated in 2-3 additional cycles.

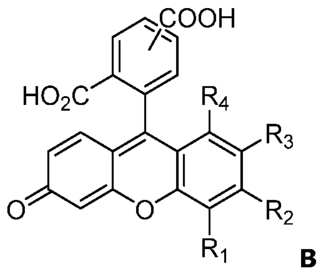
Aspect 6. The method according to any one of aspects 1, 3-5, wherein compound **5** or compound **6** is subsequently reacted with a compound of the formula **A**



A

wherein R_1, R_2, R_3 and R_4 are independently selected from hydrogen; halogen; hydroxyl; nitro; cyano; mercapto; -O-C_{1-3}-alkyl; -S–C_{1-3}-alkyl; cyclopropyl; -C_{1-3}-alkyl; -C_{2-3}-alkenyl; or -C_{2-3}-alkynyl; which -O-C_{1-3}-alkyl, -S–C_{1-3}-alkyl, cyclopropyl, -C_{1-3}-alkyl, -C_{2-3}-alkenyl or -C_{2-3}-alkynyl is optionally substituted with at least one substituent selected from halogen, hydroxyl, nitro, cyano and mercapto; with the additional option that any of the substituent pairs, R_1/R_2, R_2/R_3 and R_3/R_4 together with the intervening atoms may form an optionally substituted aromatic ring or ring system;

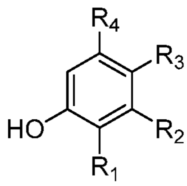
in the presence of a strong acid (e.g. methanesulfonic acid) so as to provide a compound of formula **B**



B

wherein R_1, R_2, R_3 and R_4 are as defined above.

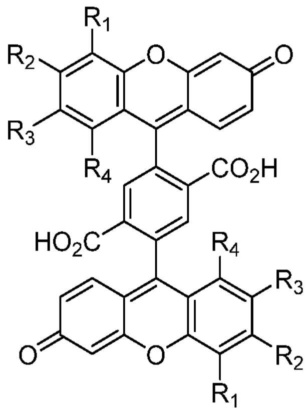
Aspect 7. The method according to any one of aspects 2-5, wherein compound **13** is subsequently reacted with a compound of the formula **A**



A

wherein R_1, R_2, R_3 and R_4 are independently selected from hydrogen; halogen; hydroxyl; nitro; cyano; mercapto; -O-C_{1-3}-alkyl; -S–C_{1-3}-alkyl; cyclopropyl; -C_{1-3}-alkyl; -C_{2-3}-alkenyl; or -C_{2-3}-alkynyl; which -O-C_{1-3}-alkyl, -S–C_{1-3}-alkyl, cyclopropyl, -C_{1-3}-alkyl, -C_{2-3}-alkenyl or -C_{2-3}-alkynyl is optionally substituted with at least one substituent selected from halogen, hydroxyl, nitro, cyano and mercapto; with the additional option that any of the substituent pairs, R_1/R_2, R_2/R_3 and R_3/R_4 together with the intervening atoms may form an optionally substituted aromatic ring or ring system;

in the presence of a strong acid (e.g. methanesulfonic acid) so as to provide a compound of formula **C**



C.

wherein R_1, R_2, R_3 and R_4 are as defined above.

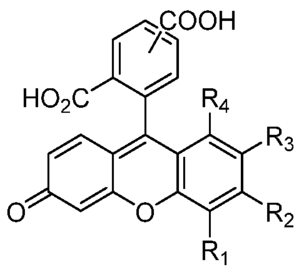
Aspect 8. The method according to any one of aspects 6-7, wherein R_2 and/or R_4 is independently hydroxyl.

Aspect 9. The method according to any one of aspects 6-8, wherein R_1 is halogen, preferably F or Cl.

Aspect 10. The method according to any one of aspects 6-9, wherein R_3 is preferably-O-C_{1-3}-alkyl, such as -OCH_3 or -OC_2H_5.

Aspect 11. The method according to any one of aspects 6-10, wherein A is a dihydroxynaphthalene, preferably 1,3-dihydroxynaphthalene, 2,3-dihydroxynaphthalene, 2,6-dihydroxynaphthalene, 1,4-dihydroxynaphthalene, 1,5-dihydroxynaphthalene, 1,6-dihydroxynaphthalene, 1,8-dihydroxynaphthalene, 1,2-dihydroxynaphthalene, 2,7-dihydroxynaphthalene or 1,7-dihydroxynaphthalene.

Aspect 12. A compound of formula B*

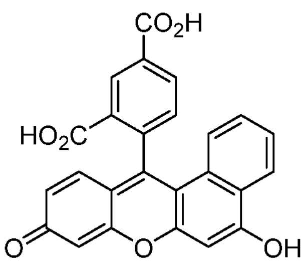


B*

wherein R_1, R_2, R_3 and R_4 are independently selected from hydrogen; halogen; hydroxyl; nitro; cyano; mercapto; -O-C_{1-3}-alkyl; -S–C_{1-3}-alkyl; cyclopropyl; -C_{1-3}-alkyl; -C_{2-3}-alkenyl; or

-C_{2-3}-alkynyl; which -O-C_{1-3}-alkyl, -S–C_{1-3}-alkyl, cyclopropyl, -C_{1-3}-alkyl, -C_{2-3}-alkenyl or -C_{2-3}-alkynyl is optionally substituted with at least one substituent selected from halogen, hydroxyl, nitro, cyano and mercapto; with the additional option that any of the substituent pairs, R_1/R_2, R_2/R_3 and R_3/R_4 together with the intervening atoms may form an optionally substituted aromatic ring or ring system.

Aspect 13. A compound according to aspect 12, having the structural formula:



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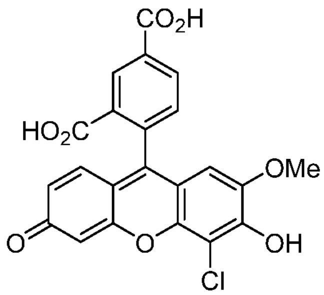

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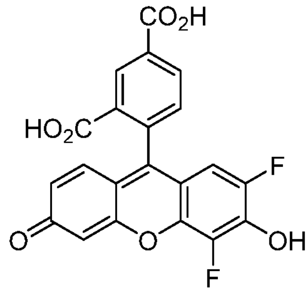
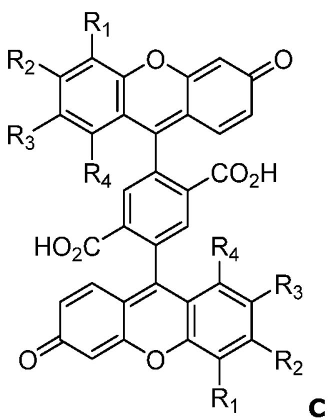


11

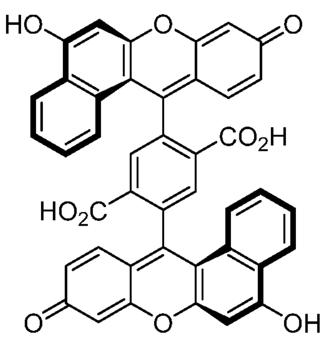
Aspect 14. A compound of formula **C***



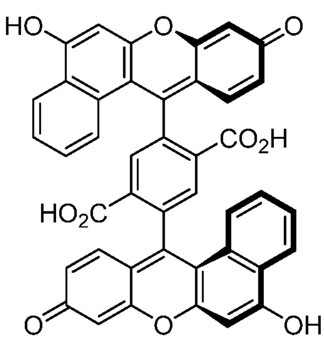
C*

wherein R_1, R_2, R_3 and R_4 are independently selected from hydrogen; halogen; hydroxyl; nitro; cyano; mercapto; -O-C_{1-3}-alkyl; -S–C_{1-3}-alkyl; cyclopropyl; -C_{1-3}-alkyl; -C_{2-3}-alkenyl; or -C_{2-3}-alkynyl; which -O-C_{1-3}-alkyl, -S–C_{1-3}-alkyl, cyclopropyl, -C_{1-3}-alkyl, -C_{2-3}-alkenyl or -C_{2-3}-alkynyl is optionally substituted with at least one substituent selected from halogen, hydroxyl, nitro, cyano and mercapto; with the additional option that any of the substituent pairs, R_1/R_2, R_2/R_3 and R_3/R_4 together with the intervening atoms may form an optionally substituted aromatic ring or ring system.

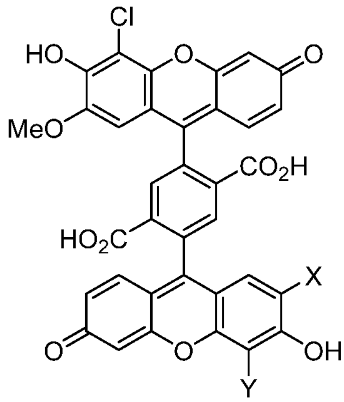
Aspect 15. A compound according to aspect 14, having the structural formula:



16 *syn*



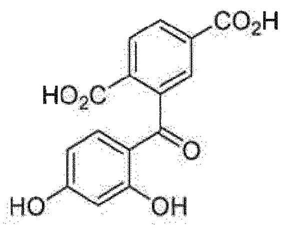
16 *anti*



17 X = Y = H
18 X = OMe, Y = Cl

CLAIMS

1. A method for the preparation and isolating of compound **6** and, optionally, of compound **5**



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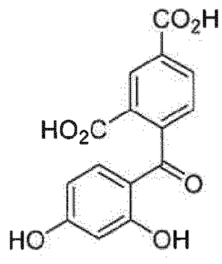


6

said method comprising the steps of:

(i) providing a condensation product mixture, being the result of a condensation reaction between trimellitic anhydride and resorcinol mediated by acid;

(ii) hydrolysing said condensation product mixture with a strong aqueous base at pH at least 11;

(iii) acidifying the reaction mixture of step (ii) so as to isolate a mixture of compound **5** and compound **6**;

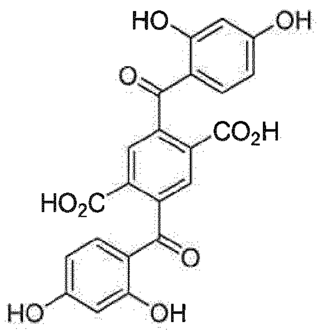
(iv) dissolving the mixture of compound **5** and compound **6** in methanol and adding water so as to precipitate compound **6**;

(v) extracting the mother liquor with an organic solvent so as to isolate compound **5** and any remaining compound **6**, and removing the organic solvent so as to obtain a dried extract;

(vi) optionally repeating steps (iv) and (v) in one or more additional cycles using the dried extract obtained in step (v);

(vii) optionally dissolving the dried extract obtained in step (v) in refluxing H_2O and precipitating compound **5**.

2. A method for the preparation and isolation of compound **13**



13

said method comprising the steps of:

(i) providing a condensation product, being the result of a condensation reaction between pyromellitic dianhydride and resorcinol mediated by acid;

(ii) hydrolysing said condensation product with a strong aqueous base at pH of at least 11;

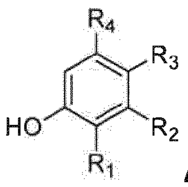
(iii) acidifying the reaction mixture of step (ii) so as to isolate compound **13**.

3. The method according to any one of claims 1-2, wherein hydrolysis steps (step ii.) are carried out at a pH of 12-14, preferably using a 1:1 weight ratio mixture of NaOH and H_2O.

4. The method according to any one of claims 1-3, wherein the acidification steps (step iii) are carried out using 12 M HCl.

5. The method according to any one of claims 1, 3 or 4, wherein, in step vi, steps (iv) and (v) are repeated in 2-3 additional cycles.

6. The method according to any one of claims 1, 3-5, wherein compound **5** or compound **6** is subsequently reacted with a compound of the formula **A**

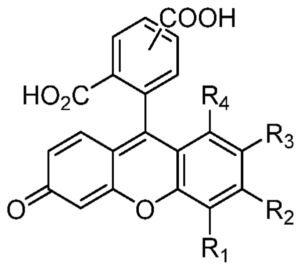


A

wherein R_1, R_2, R_3 and R_4 are independently selected from hydrogen; halogen; hydroxyl; nitro; cyano; mercapto; -O-C_{1-6}-alkyl; -S-C_{1-6}-alkyl; cyclopropyl; -C_{1-6}-alkyl; -C_{1-6}-alkyl-

CONH-R_5, -C_{2-6}-alkenyl; or -C_{2-6}-alkynyl; which -O-C_{1-6}-alkyl, -S–C_{1-6}-alkyl, cyclopropyl, -C_{1-6}-alkyl, -C_{2-6}-alkenyl or -C_{2-6}-alkynyl is optionally substituted with at least one substituent selected from halogen, hydroxyl, -COOH, nitro, cyano and mercapto; wherein R_5 is selected from the group consisting of -C_{1-6}-alkyl and -[CH_2CH_2O]$_n$, wherein n=1-10,000, wherein said -C_{1-6}-alkyl and -[CH_2CH_2O]$_n$ are optionally substituted with a substituent selected from the group consisting of -NH-biotin, -C_{1-6}-alkyl-heterocycloalkyl, -DOTA, -NHCO-C_{1-6}-alkyl-heterocycloalkyl, -maleimide, -N_3, -C≡CH, -C-$_{1-6}$-alkyl-N_3, and -C-$_{1-6}$-alkyl-N(-C-$_{1-6}$-alkyl-heteroaryl)$_2$; with the additional option that any of the substituent pairs, R_1/R_2, R_2/R_3 and R_3/R_4 together with the intervening atoms may form an optionally substituted aromatic ring or ring system;

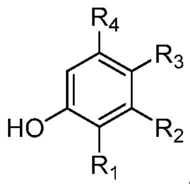
in the presence of a strong acid (e.g. methanesulfonic acid) so as to provide a compound of formula **B**



B

wherein R_1, R_2, R_3 and R_4 are as defined above.

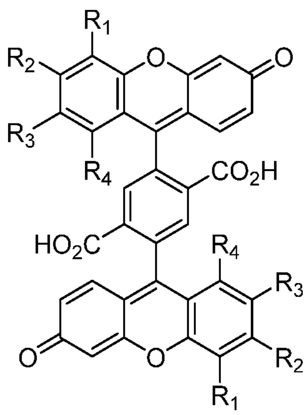
7. The method according to any one of claims 2-5, wherein compound **13** is subsequently reacted with a compound of the formula **A**



A

wherein R_1, R_2, R_3 and R_4 are independently selected from hydrogen; halogen; hydroxyl; nitro; cyano; mercapto; -O-C_{1-6}-alkyl; -S–C_{1-6}-alkyl; cyclopropyl; -C_{1-6}-alkyl; -C_{1-6}-alkyl-CONH-R_5, -C_{2-6}-alkenyl; or -C_{2-6}-alkynyl; which -O-C_{1-6}-alkyl, -S–C_{1-6}-alkyl, cyclopropyl, -C_{1-6}-alkyl, -C_{2-6}-alkenyl or -C_{2-6}-alkynyl is optionally substituted with at least one substituent selected from halogen, hydroxyl, -COOH, nitro, cyano and mercapto; wherein R_5 is selected from the group consisting of -C_{1-6}-alkyl and -[CH_2CH_2O]$_n$, wherein n=1-10,000, wherein said -C_{1-6}-alkyl and -[CH_2CH_2O]$_n$ are optionally substituted with a substituent selected from the group consisting of -NH-biotin, -C_{1-6}-alkyl-heterocycloalkyl, -DOTA, -NHCO-C_{1-6}-alkyl-heterocycloalkyl, -maleimide, -N_3, -C≡CH, -C-$_{1-6}$-alkyl-N_3, and -C-$_{1-6}$-alkyl-N(-C-$_{1-6}$-alkyl-heteroaryl)$_2$; with the additional option that any of the substituent pairs, R_1/R_2, R_2/R_3 and R_3/R_4 together with the intervening atoms may form an optionally substituted aromatic ring or ring system;

in the presence of a strong acid (e.g. methanesulfonic acid) so as to provide a compound of formula **C**



C.

wherein R_1, R_2, R_3 and R_4 are as defined above.

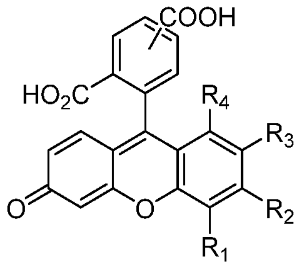
8. The method according to any one of claims 6-7, wherein R_2 and/or R_4 is independently hydroxyl.

9. The method according to any one of claims 6-8, wherein R_1 is halogen, preferably F or Cl.

10. The method according to any one of claims 6-9, wherein R_3 is -O-C_{1-3}-alkyl, such as -OCH_3 or -OC_2H_5, or -C_{1-3}-alkyl substituted by –COOH, such as –C_2-alkyl substituted by –COOH.

11. The method according to any one of claims 6-10, wherein A is a dihydroxynaphthalene, preferably 1,3-dihydroxynaphthalene, 2,3-dihydroxynaphthalene, 2,6-dihydroxynaphthalene, 1,4-dihydroxynaphthalene, 1,5-dihydroxynaphthalene, 1,6-dihydroxynaphthalene, 1,8-dihydroxynaphthalene, 1,2-dihydroxynaphthalene, 2,7-dihydroxynaphthalene or 1,7-dihydroxynaphthalene.

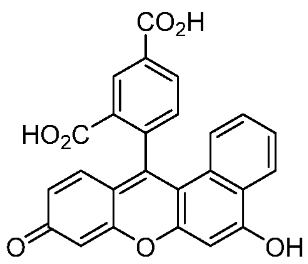
12. A compound of formula B*



B*

wherein R_1, R_2, R_3 and R_4 are independently selected from hydrogen; halogen; hydroxyl; nitro; cyano; mercapto; -O-C_{1-6}-alkyl; -S–C_{1-6}-alkyl; cyclopropyl; -C_{1-6}-alkyl; -C_{1-6}-alkyl-CONH-R_5, -C_{2-6}-alkenyl, or -C_{2-6}-alkynyl; which -O-C_{1-6}-alkyl, -S–C_{1-6}-alkyl, cyclopropyl, -C_{1-6}-alkyl, -C_{2-6}-alkenyl or -C_{2-6}-alkynyl is optionally substituted with at least one substituent selected from halogen, hydroxyl, -COOH, nitro, cyano and mercapto; wherein R_5 is selected from the group consisting of -C_{1-6}-alkyl and -$[CH_2CH_2O]_n$, wherein n=1-10,000, wherein said -C_{1-6}-alkyl and -$[CH_2CH_2O]_n$ are optionally substituted with a substituent selected from the group consisting of -NH-biotin, -C_{1-6}-alkyl-heterocycloalkyl, -DOTA, -NHCO-C_{1-6}-alkyl-heterocycloalkyl, -maleimide, -N_3, -C≡CH, -C-$_{1-6}$-alkyl-N_3, and -C-$_{1-6}$-alkyl-N(-C-$_{1-6}$-alkyl-heteroaryl)$_2$; with the additional option that any of the substituent pairs, R_1/R_2, R_2/R_3 and R_3/R_4 together with the intervening atoms may form an optionally substituted aromatic ring or ring system, with the proviso that when R_1 = R_3 = R_4 = hydrogen, then R_2 is different from hydroxyl.

13. A compound according to claim 12, having the structural formula:



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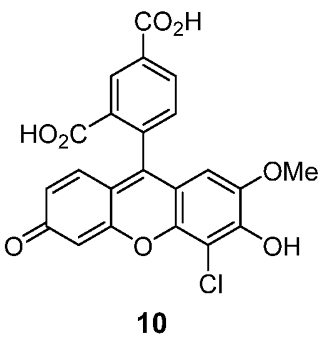

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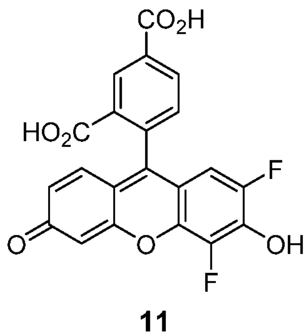

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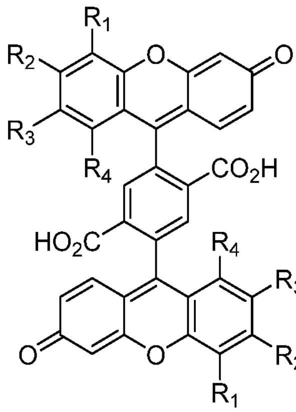
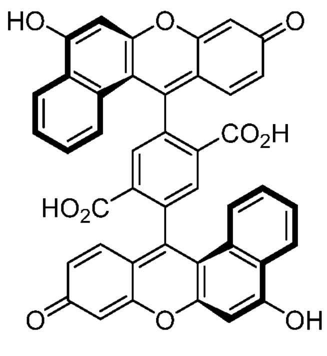
14. A compound of formula **C***



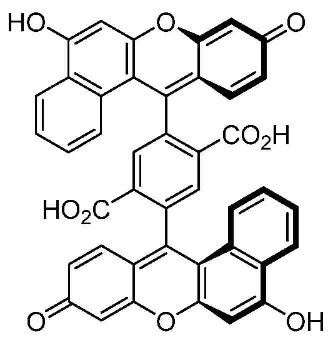
C*

wherein R_1, R_2, R_3 and R_4 are independently selected from hydrogen; halogen; hydroxyl; nitro; cyano; mercapto; -O-C_{1-6}-alkyl; -S–C_{1-6}-alkyl; cyclopropyl; -C_{1-6}-alkyl; -C_{1-6}-alkyl-CONH-R_5, -C_{2-6}-alkenyl; or -C_{2-6}-alkynyl; which -O-C_{1-6}-alkyl, -S–C_{1-6}-alkyl, cyclopropyl, -C_{1-6}-alkyl, -C_{2-6}-alkenyl or -C_{2-6}-alkynyl is optionally substituted with at least one substituent selected from halogen, hydroxyl, -COOH, nitro, cyano and mercapto; wherein R_5 is selected from the group consisting of -C_{1-6}-alkyl and -$[CH_2CH_2O]_n$, wherein n=1-10,000, wherein said -C_{1-6}-alkyl and -$[CH_2CH_2O]_n$ are optionally substituted with a substituent selected from the group consisting of -NH-biotin, -C_{1-6}-alkyl-heterocycloalkyl, -DOTA, -NHCO-C_{1-6}-alkyl-heterocycloalkyl, -maleimide, -N_3, -C≡CH, -C-$_{1-6}$-alkyl-N_3, and -C-$_{1-6}$-alkyl-N(-C-$_{1-6}$-alkyl-heteroaryl$)_2$; with the additional option that any of the substituent pairs, R_1/R_2, R_2/R_3 and R_3/R_4 together with the intervening atoms may form an optionally substituted aromatic ring or ring system, with the proviso that when $R_1 = R_3 = R_4$ = hydrogen, then R_2 is different from hydroxyl.

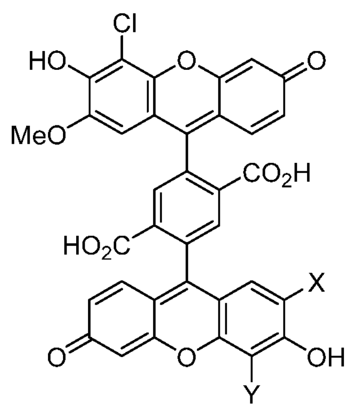
15. A compound according to claim 14, having the structural formula:



16 *syn*

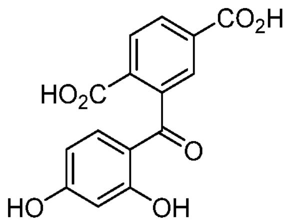


16 *anti*



17 X = Y = H
18 X = OMe, Y = Cl

16. A compound of the formula 5 or 6



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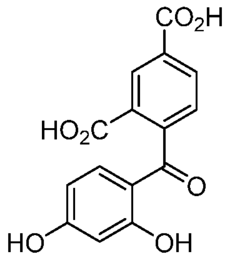

6.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/059950

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07C65/40 C07D311/82 C07D407/10 C07C51/377 C07C51/43
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07C C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2002/146726 A1 (MATRAY TRACY [US] ET AL) 10 October 2002 (2002-10-10) abstract figure 1; compounds AMD008, AMD009 pages 22-23; compounds 8, 9 pages 1-52 ----- -/--	12,13 1-11, 14-16

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 August 2015	13/08/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Dunet, Guillaume

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YUICHIRO UENO ET AL: "Preparation of 5- and 6-Carboxyfluorescein", SYNTHESIS, vol. 2004, no. 15, January 2004 (2004-01), pages 2591-2593, XP055136212, ISSN: 0039-7881, DOI: 10.1055/s-2004-829194	12
Y	abstract	13-16
A	* scheme 1 *; page 2591 page 2591; figure 1 page 2592, right-hand column, paragraph 2	1-11

X	CN 103 012 354 A (GUANGZHOU GENERAL PHARMACEUTICAL RES INST) 3 April 2013 (2013-04-03)	12
Y	abstract	13-16
A	* scheme *; page 3 claims 1-4	1-11

X	US 4 945 171 A (HAUGLAND RICHARD P [US] ET AL) 31 July 1990 (1990-07-31)	12
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